AMYLOID SUBSTANCE AND AMYLACEOUS BODIES IN MULTIPLE SYPHILITIC TUMORS OF THE BONES, WITH REMARKS ON THE RELATION OF AMYLACEOUS BODIES TO AMYLOID SUBSTANCE.

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Plates VI and VII.

For the pathological material utilized in the following report I am indebted to the courtesy of Drs. L. Newmark and P. K. Brown of San Francisco. The clinical history of the case, which presents some interesting features from a neurological point of view, will be published *in extenso* by Dr. Newmark. In so far as regards our present report it will suffice to say that we learn from it, that 25 years before he came under the charge of Dr. Newmark, the patient suffered from an ulceration of the penis, as evidence of which there remained a large scar. Whether any typical secondary symptoms ever manifested themselves could not be established with absolute certainty. An energetic antisyphilitic treatment, which was given at once, proved to be without any immediate beneficial result.

The findings at autopsy were the following:

Autopsy about 24 hours after death. Strongly built, somewhat emaciated man of middle age. Marked œdema of lower extremities and lower part of trunk; slight œdema of scrotum and penis. Intense cyanosis of face and neck.

On right side of thorax in the axillary line about 15 cm. below axilla is an oblique incision, 4 cm. in length. The bottom of the wound is covered with soft red tissue resembling healthy granulations. In the skin in the neighborhood of the wound, as well as in a number of places on the back, dark purple, almost black nodules of the size of millet-seeds or a little larger are observed, which on incision present the appearances of cutaneous hæmorrhages, but are rather distinctly outlined. There

is slight round kyphosis of the upper dorsal and compensatory lordosis of the cervical spine. The processus spinosi of the 2nd and 3rd dorsal vertebræ are infiltrated with reddish-brown, soft masses of tumor. There is a considerable quantity of similar material between the bone and dura mater at this point. The dura mater itself is normal. The infiltration extends far into the bodies of the 2nd and 3rd dorsal vertebræ. The spinal cord is markedly compressed and softened. Ascites, hydrothorax, and hydropericardium are present.

Heart.—Twice the normal size. All cavities equally dilated. Walls of both ventricles thicker than normal (wall of right ventricle averages 5 mm., that of left ventricle about 12 mm.). Valves intact. A few yellow slightly raised spots in the intima of aorta and coronary arteries. The heart muscle is dark brown, very translucent, of waxy appearance. It is extremely firm, so much so that after the opening of the cavities and removal of their contents the heart does not collapse, the walls retaining exactly their former position and shape.

Lungs.—Both apices adherent. From the points of adhesion pigmented scars extend for some distance into the pulmonary tissue. On both sides the visceral and to a greater extent the parietal pleuræ are transformed into thick layers of dense white scar-tissue with smooth glistening inner surfaces. Both lower lobes are entirely collapsed; the middle lobe and both upper lobes partly so.

A few lymph glands in the lower anterior mediastinum are enlarged, some of them to the size of small beans. They are very hard to the touch. Cut surface smooth and of a greyish-brown color. Tissue slightly translucent. All of them are surrounded by dense scar-tissue.

Several *ribs* on both sides of the body show large fusiform swellings, some of them as large as goose-eggs. The enlargement is noticeable on all sides but is more marked on the pleural surface, where some of them project from 2-3 cm. above the normal level of the inner surface of the chest wall. On incision they prove to be due to the presence of a reddish-brown, almost pulpy material inside the bone, which has more or less completely replaced the normal structures. The centre of these tumors is darker, the periphery lighter and more translucent, having a waxy appearance. On the outside they, for the most part, disappear gradually into the adjoining healthy tissue, but in some places a somewhat sharper outline is formed by a row of thin spicula of bone. The tumors themselves do not contain any bone. On the inside all of them are covered by the thickened pleura.

Spleen.—One and a half times the normal size; very hard. Cut sur-

face dark purple, smooth. Malpighian bodies visible as greyish-white, translucent dots of the size of a large pin's head. Trabeculæ normal.

Both *adrenals* contain little fat; otherwise they present nothing abnormal.

Both *kidneys* are of normal size; capsule slightly adherent; after its removal the surface appears somewhat granular. The tissue is very hard. Cut surface dark purple; cortex a little cloudy. Glomeruli plainly visible as greyish-red dots. Both renal pelves normal.

Liver.—Small, very hard. Cut surface dark purple; lobules small; centre dark purple; retracts a little from cut surface; periphery lighter.

Pancreas.—In the tail a greyish-red softened area of the size of a hazelnut is observed.

Slight enlargement of mesenteric and retroperitoneal lymph-glands; cut surface pale greyish-white.

In the region between the body and manubrium the *sternum* shows an ovoid enlargement which is more marked on the right side. A cut through the centre of the thickening shows it to be caused by an ovoid tumor, measuring 3 x 1.5 cm. in diameter. The centre of the tumor has a dark, reddish-brown color, the periphery is lighter, greyish-brown, waxy. The entire mass consists of soft, pulpy tissue without any bone. On all sides the tumor is surrounded by a thin shell of bone.

No marked changes in any of the other organs.

Microscopic examination gives the following results: The softening of the pancreas is due to post-mortem digestion. The kidneys exhibit signs characteristic of an old interstitial nephritis with more recent inflammatory changes in the vicinity of some of the medium-sized subcapsular veins. In spots the epithelium of the convoluted tubules is in a condition of fatty degeneration: in others it is necrotic and partly desquamated. The majority of the renal tubules are filled with granular material or hyaline casts. Many of the vascular loops of the glomeruli and all the medium-sized and small arteries contain amyloid substance in their walls. Many of the arteries show an additional irregular thickening of the intima.

The purple spots in the skin prove to be hæmorrhages into the cutis.

The heart muscle contains large quantities of amyloid substance. The larger part of it is found in the walls of blood-vessels. In those with a muscular coat the outer layers of the media are more particularly affected. A considerable amount of amyloid is found also in the endo- and peri-mysium. Many of the muscle cells are surrounded by a more or less circular sheath of amyloid, which in enlarging has caused first

atrophy, then complete destruction of the cells. By this process several small areas have been completely deprived of muscle cells.

Sections of tissue hardened in 10% aqueous solution of formalin, when tested for amyloid substance, give the following reactions: dilute solution of iodine, no reaction (perhaps due to the hardening fluid); iodine sol. and sulphuric or oxalic acid (5%), bluish-green reaction. Methyl-violent stains the material rose pink.

Enlarged lymph glands from different parts of the body show under the microscope the signs of a chronic catarrhal inflammation with beginning proliferation of connective tissue in the lymph sinuses.

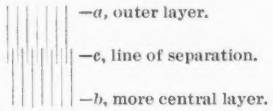
In describing the microscopic appearances of the *sternal tumor* it is best to distinguish 3 layers, a central, a peripheral and an intermediate zone.

1. *Peripheral zone.*—The peripheral layer is rich in cells, with but few connective-tissue fibres between them. The majority of the cells are small and possess round, intensely staining nuclei (Plate VI, Fig. 1). Their close resemblance to lymphocytes and in some instances to plasma cells is striking. In addition to such forms, but fewer in number, we find large cells with vesicular nuclei of oval or more irregular shape. The bodies of the latter are sometimes fusiform (Plate VI, Fig. 1, b), but mostly spherical or polymorphous. The protoplasm composing them is partly granular, but in many instances of a more homogeneous nature (Plate VI, Fig. 1, c; Fig. 2, a). These larger cells have a tendency to coalesce. In a number of such conglomerations the nuclei, and sometimes even the outlines of the cells composing them, are more or less plainly visible (Plate VI, Fig. 1, d; Fig. 2, b). As indicated in the drawings not all the nuclei found in them are vesicular, but some of them are of the lymphocytic type, which shows that the smaller cells also, though to a minor degree, participate in their formation. Eventually the nuclei disappear and there remain irregular, more or less homogeneous masses, some of which are filled with nuclear debris. Around these central masses as a nucleus new material has been deposited in concentric layers and in this way bodies have been formed which in sections, as well as when isolated by teasing, resemble morphologically in every detail amylaceous bodies.

This resemblance is not confined to their external appearance, as is proved by the way in which they react to iodine or methyl-violet. In dilute iodine-solution (e. g. Gram's solution), they stain a dark mahogany brown; the color is generally more intense in the central nucleus than in the peripheral layers. When sulphuric acid is added, there

is, at least in the isolated bodies, no very perceptible change in color, even after 24 hours and more. Methyl-violet stains them a dark blue, which takes a slight purplish tinge within 24 hours. When thin sections of hardened tissue (10% formalin) are first immersed in 5% solution of sulphuric acid and then in Gram's solution the concentric layers in the periphery take a dark brown color, whereas the irregular nucleus shows a bluish-green color. In a similar manner we find that in sections methyl-violet gives the peripheral layers a blue and the central "nucleus" a purple color.

The resemblance to ordinary amylaceous bodies, therefore, seems to be close enough to identify them as such. In very thin sections (4-6 μ) which are stained with methyl-violet and mounted in 20% aqueous solution of acetate of potash, the oil immersion permits the recognition of one more important detail in the structure of the peripheral concentric layers, which is not visible in specimens mounted in Canada balsam. It is then seen, that these layers show most perfect, very closely set, radial markings. Plate VII, Fig. 7, gives only a very inadequate idea of their regularity and delicacy. These markings are the dividing lines between innumerable, very minute, parallel, closely packed needles, of which the concentric layers consist. That they really are needles, and not delicate lamellæ, was shown in one place, where a tangential section had carried off the extreme edge of one of the amylaceous bodies. Here the needles of course appeared, on cross section, as just visible, dark blue dots. In spite of persistent endeavors I have not been able to isolate these needles. They seem to adhere rather firmly to one another and their extreme minuteness in itself would naturally render it difficult to separate them from one another. But even when isolated it would most likely be impossible to make out much about their shape in its finer details. Between the different layers of needles there are darker blue concentric lines, which separate them from one another. These probably represent the tips of the needles from the inner layers reaching in between the bases of those of the peripheral layer somewhat in the way represented in the following diagram:



c naturally appears as a dark line because here the needles are more densely packed together than in *a* and *b*.

The needles of the superficial layers are not all of exactly the same

length. The surfaces of the bodies are, therefore, somewhat uneven, or present a prickly appearance, a fact which may account to some extent for the formation of large giant cells (Plate VII, Fig. 5, a) in their neighborhood. It would seem most probable that these enormous giant cells, which sometimes almost completely envelop the amylaceous bodies, do not furnish any new substance for their enlargement, but on the contrary destroy them. Such an assumption would at least account for the fact that they are frequently situated in excavations in the surface of the amylaceous bodies, so that they would be analogous to the osteoclasts in Howship's lacunæ and would furnish another illustration of the destructive influence of these foreign-body giant cells (Fremdkörperriesenzellen) upon the material around which they develop.

The "nucleus" of the amylaceous bodies, which stains purple with methyl-violet, is generally more lumpy in character, but in some places even this part of the amylaceous body is found to be composed of densely packed needles, which are arranged in a radial fashion around a central point (Plate VII, Fig. 7, a). In these cases the crystalline needles also stain purple in methyl-violet.

Crystalline formations are not found exclusively in the amylaceous bodies. In a few spots I have encountered isolated large bunches of needles arranged in a radial fashion around a more homogeneous central body; needles and nucleus giving the amyloid reaction with methyl-violet

Forms represented in Plate VII, Fig. 8, are even more peculiar. In the centre or more towards the periphery of an irregular lump of colloid substance which takes a blue color in methyl-violet, we find a spherical or ovoid cavity containing a rosette of purple needles.

Plate VI, Fig. 4, shows another peculiarity of the amylaceous bodies, which is very marked in our case. They frequently become adherent to one another and in this way large conglomerations consisting of 20 or more of these bodies are formed.

There remains to be discussed briefly one point of some interest and that is: At what stage in the transformation of the conglomerated large cells into the nucleus of amylaceous bodies does the amyloid reaction appear? The specimens show in this regard that the transition of the colloid protoplasm into amyloid material is a slow and gradual one. Even before the nuclei have disappeared, the cell bodies begin to stain a little darker yellow than the surrounding tissue and many of them also show a faint but quite distinct purple color when treated with methyl-violet.

The blood-vessels in the peripheral layers of the tumor are few in number, of small calibre, and their walls are normal.

The peripheral cellular layers, which I have just described and in which the amylaceous bodies are formed, do not form a regular, even zone in the periphery of the tumor, but are present in irregular patches, most of them rather limited in extent, which invariably, however, are found in the periphery.

2. *Intermediate zone.*—The tissue loses to a great extent its cellular character and shows the appearance represented in Plate VII, Fig. 5. It consists of a fine meshwork of connective tissue, the spaces of which are filled with numerous amylaceous bodies isolated or arranged in groups. The larger part of them are surrounded by large giant cells. In some spots in the connective tissue there is an abundant deposit of brown pigment (Fig. 5, b).

In the intermediate zone of the tumor we find, in addition to the amylaceous bodies, thick irregular bands and lumps of ordinary amyloid substance, which appear first in the reticulum of connective tissue. When stained with methyl-violet these bands mostly take a dark purple color throughout, but in all sections are some in which the purple centre is surrounded by a dark blue colloid material.

3. The *centre* of the tumor is composed of fragments of amylaceous bodies and of amyloid bands. The spaces between them are filled with red blood corpuscles. In some places, among the masses of red blood corpuscles, it is possible to follow out for some distance collapsed tubes, with their endothelial walls, which apparently are partly destroyed capillaries that have discharged their contents into the surrounding parts.

All the colloid material in the central parts of the tumor stains with dilute iodine solution brown, which turns to a bluish-green when sulphuric acid is added. With methyl-violet it takes a purple color.

The formation of amyloid substance is not confined to the tumor itself, but is also noticed, although to a minor degree, in the neighboring tissues. Plate VII, Fig. 6, for example, shows a portion of the periosteum under the higher power. The amyloid material is seen to be deposited in and between the bundles of fibres of connective tissue and also in the walls of capillaries. The specimen from which the drawing was taken was stained by Van Gieson's method,[1] which, as Schmidt [2] has pointed out, brings out clearly the relation of the connective-tissue fibres

[1] In the drawing the bright purple connective-tissue fibres are shown as black lines.

[2] "Ueber die localen Amyloidtumoren der Zunge," Virchow's *Archiv*, 1896, cxliii, p. 369.

to the amyloid material. A careful study of this specimen and of many others of the same kind shows that nowhere is there the slightest indication of a transformation of connective-tissue fibres into amyloid substance. The latter always appears first in the spaces between the fibres, it thrusts them apart and destroys them, at least to a certain extent, as may be concluded from the presence of short pieces of apparently broken fibres in some of the amyloid bands. There is, however, no evidence of a direct transformation of one into the other.

Not only amyloid substance proper but also a few amylaceous bodies may be found in the tissues, sometimes in the neighborhood of the tumor, but at other times quite remote from it.

Specimens of the tumor of the spinal column which were examined with the microscope had precisely the same appearance as the intermediate zone of the sternal tumor.

The costal tumors also differ very little from the sternal one. The number of amylaceous bodies in them is smaller, but there is comparatively more ordinary amyloid substance and also more fibrous but very little cellular tissue.

Unfortunately it was impossible to make the customary tests for amyloid at the time of the autopsy, which was performed at an undertaker's establishment under unfavorable conditions. By mistake material for microscopic examination was not obtained from the spleen or liver. Nevertheless, the macroscopic appearance of the spleen indicated the presence of amyloid in this organ. In regard to the liver, which showed evidence of a certain degree of cyanotic atrophy, I am doubtful about this point.

The findings, therefore, may be summarized as follows:

Multiple tumors of bones (sternum, ribs, spinal column) with local formation of amyloid substance and amylaceous bodies in them and in the surrounding tissues; amyloid degeneration of kidneys, heart and most likely also of spleen; interstitial and parenchymatous nephritis; hydrothorax, ascites, hydropericardium, anasarca, chronic passive congestion of the abdominal viscera.

In considering the post-mortem findings, first of all the question arises: What is the nature of these tumors?

In looking over the literature at my disposal I find that Hildebrand[3] has described a tumor, which, at least in some respects, re-

[3] Virchow's *Archiv*, 1895, cxl, p. 249.

sembles very closely those found in our case. The tumor, which was examined by him, was situated in the sternum. It had been removed by König with good results, although at the operation two serous cavities—one pleura and the pericardium certainly, and very likely also the other pleura—were opened. The patient died later at home of a disease apparently not connected with the former presence of the tumor. No autopsy could be obtained. The tumor was of the size of a prune. It did not have any bony capsule, nor was there any bone in the tumor itself. The histological examination revealed the presence of a richly cellular tissue composed mainly of two forms of cells—smaller ones with intensely staining nuclei, and larger ones which took the nuclear stains more faintly. The protoplasm of the latter was granular and stained well with eosin. There were also some giant cells. Some connective-tissue fibres could be made out between the cells. The capillaries were large and numerous. A large number of amylaceous bodies and a good deal of amyloid material in bands and lumps were present in the cellular tissue. The very intimate relation between amylaceous bodies and adjoining cells, which was observed in some spots, led Hildebrand to infer that the amylaceous bodies originated, partly at least, from degenerating cells of the tumor. There is not even an allusion to any syphilitic history either in König's or in Hildebrand's report; presumably therefore there was nothing that pointed to the existence of such an infection. On the basis of his microscopic findings Hildebrand regards his tumor as a myelogenic giant-celled sarcoma (endostales Riesenzellensarcom). But in spite of the great general similarity which exists even in the microscopic findings, in both cases, I am doubtful whether to accept Hildebrand's diagnosis, at least in our case. When we compare the microscopic findings one important difference at once becomes apparent, and that is the presence of a considerable quantity of ordinary connective tissue at least in the inner, and therefore most likely older, layers of our tumors. This characterizes, to my mind conclusively, the tissue composing them as granulation tissue and excludes the possibility of their being sarcomatous. It is possible that Hildebrand was misled in his diagnosis by the presence of only the earliest stages

of granulation tissue in his sections. But this suspicion could not of course be verified without a renewed study of sections from his tumor.

An interesting feature of our case, undoubtedly, is the possibility offered of tracing with comparative ease the mode of formation of the amylaceous bodies present. When we study the literature in regard to their formation in other localities of the body, we find very marked discrepancies between the statements of different writers. In fact the divergences are so striking that one might be inclined to believe that the *modus formandi* is not the same in all localities and may differ even in the same organ according to circumstances.

The material composing these bodies might be furnished in any of three ways: (1) By degeneration of cells or formed intercellular substance; (2) by precipitation from the tissue juices, or (3) by a combination of (1) and (2), part of the material being furnished by degeneration, and part of it by precipitation from fluids.

The second view, viz., that the bodies are merely the result of precipitation from the surrounding fluids, has been frequently connected with the names of Virchow and of Rindfleisch, although in their writings, so far as they have been at my disposal, I have been unable to find any direct statement to this effect. Friedreich[4] on the other hand undoubtedly regarded the amylaceous bodies, which he discovered in the lungs, as such precipitations. He believed that they were formed from extravasated blood by a peculiar form of coagulation.

More or less in favor of a cellular origin of amylaceous bodies are Ziegler,[5] Stilling[6] (these two at least so far as prostatic concretions are concerned), Zahn,[7] Favre,[8] and Wichmann[9] (the last three in a more general way). Ziegler and Wichmann are of the opinion that in the prostate the epithelial cells of the glands are transformed into homogeneous masses by a degenerative process and these, or frag-

[4] Virchow's *Archiv*, 1856, x, pp. 201, 507.

[5] Lehrb. der allgem. Path. und path. Anat., 9te Aufl., i, p. 226, Jena, 1898.

[6] Virchow's *Archiv*, 1884, xcviii, p. 1.

[7] Virchow's *Archiv*, 1878, lxxii, p. 119.

[8] Favre, Thèse de Genève, 1879, cited from Siegert, Virchow's *Archiv*, 1892, cxxix, p. 517.

[9] Ziegler's *Beiträge*, 1893, xiii, p. 487.

ments of such, coalesce to form the amylaceous bodies. Among the authors that have described amylaceous bodies in tumors,[10] there are also two who believe that in their cases these bodies had originated from degenerating cells or that degeneration of cells played at least an important rôle in their formation. One of these, Hildebrand, who, as I said above, was probably led to this view by the close connection sometimes found between degenerating cells and amylaceous bodies, nevertheless could not arrive at a definite opinion as to whether the material composing them was excreted by the degenerating cells or the cells themselves were directly transformed into amylaceous bodies. Langhans,[11] who describes a carcinoma of the lung containing amylaceous bodies, believes he has seen in his specimens transitional stages between degenerating tumor cells and such bodies.[12]

Posner[13] altogether rejects an exclusive view on the subject and states as his belief that amylaceous bodies may be formed not less by degenerative processes from cells than by precipitation from fluids; while Paulizky[14] arrives at the conclusion that in the prostatic concretions the centre, the so-called "nucleus" (Friedreich[15]), is a product of degenerating cells, whereas the peripheral concentric layers are precipitations from the surrounding fluids.

The third view, that products furnished by degenerating cells and the tissue juices combine in the formation of amylaceous bodies, is held by Siegert,[16] at least for part of the concretions (corpora versicolorata). Ziegler also seems to favor it so far as the formation of amylaceous bodies in the lungs and central nervous system is concerned, while Lubarsch[17] has endorsed it in a more general way for all forms.

[10] For the literature on this subject, see Hildebrand's article, loc. cit.

[11] Virchow's *Archiv*, 1867, xxxviii, 537.

[12] Langhans mentions in his paper that amylaceous bodies were found only in the peripheral parts of the tumor. This would suggest the possibility that they were formed in the lung before it was invaded, by the carcinoma, and that they might have been taken up only later into the growing tumor.

[13] *Zeitschr. f. klin. Med.*, 1889, xvi, p. 144.

[14] Virchow's *Archiv*, 1859, xvi, 147.

[15] l. c.

[16] Virchow's *Archiv*, 1892, cxxix, p. 513.

[17] Lubarsch and Ostertag's *Ergebnisse*, Abth. II: Allg. path. Morph. u. Physiol., p. 200, Wiesbaden, 1895.

Siegert's[18] attempt to solve the difficulties by subdividing the amylaceous bodies into two groups according as they give certain color-reactions (corpora versicolorata) or not (corp. flava), and postulating a different *modus formandi* for each group, does not appear to be very successful. A subdivision on this basis was first suggested by Virchow long ago, but Lubarsch,[19] although accepting it in a general way, has pointed out some of the difficulties which we encounter, when we try to adhere to it strictly. For myself, I must confess that in studying the amylaceous bodies of the prostate it has been absolutely impossible for me to arrive at any satisfactory classification on these lines. There are so many transitional forms between bodies giving no reaction to solutions of iodine and those with typical amyloid reaction, that it has been impossible for me to find any sharp line of division between Siegert's two classes. Furthermore, when we employ diluted sulphuric acid in addition to the iodine solution, the matter becomes even more complicated, because sometimes bodies which will not react with iodine alone, turn green as soon as sulphuric acid is added, and again, part of an amylaceous body, for instance, the periphery, may show no reaction, whereas in the centre it is quite marked. Similar differences in reaction become apparent, when we use solutions of methyl-violet. On account of these difficulties I do not believe that much can be gained by so artificial a subdivision. The reactions, which at first sight seem to be so important, on closer study lose much of their apparent value and on account of the infinite variations encountered it would seem only natural to conclude that they are not essential but more or less accidental. This conviction has been much strengthened by the study of the amylaceous bodies found in the tumor-like growths in our case, because even the crystalline needles, which were present, in some places reacted to methyl-violet and in others not at all.[20]

[18] loc. cit.

[19] loc. cit.

[20] I have purposely omitted to mention the different views held concerning the formation of amylaceous bodies in the central nervous system, since they are so contradictory as to make a renewed careful study of this subject necessary before any intelligent use can be made of the data which have accumulated in course of time.

My specimens seem to leave hardly any room for doubt, that in our case the central masses of the amylaceous bodies, in a manner similar to that described by Paulizky[21] for the prostatic concretions, were largely furnished by degenerating cells, whereas the crystalline needles in the periphery are more likely to be precipitations from the surrounding fluids.

When I say degenerating cells, I do not wish to employ the word in its strictest sense. It would perhaps be better to speak of a transformation (metamorphosis) of the cells, because I believe that the colloid material, which accumulates in their bodies and which corresponds so closely to the amyloid material found in other parts of the tumors outside of cells without any connection with a degenerative process in the latter, is largely taken up into the cells from the outside. Such being the case the process has more of the nature of an infiltration, if in the light of recent knowledge we are at all justified in making such a strict distinction between degeneration and infiltration.

It seems that in our case the more irregular central masses, the "nucleus," furnished a point of crystallization for the needles on the outside, although evidence of crystallization may sometimes also be found in the central masses themselves.

The present is not the first observation of an apparent crystallization of amyloid material. Maximow[22] found in the liver of a horse, with amyloid degeneration of the viscera an amyloid substance in a crystalline form. Some of the irregular masses of amyloid material were covered with needles somewhat varying in width, which gave a typical reaction with methyl-violet. Maximow found similar needles in the livers of rabbits with experimental amyloid degeneration, although he could detect them only in specimens which were hardened in alcohol and was unable to see them in the fresh tissues. He also claims to have encountered appearances similar to these, although in a much less marked degree, in human livers with amyloid degeneration. On the strength of my own observations I am satisfied that

[21] l. c.

[22] Virchow's *Archiv*, 1898, cliii, p. 361.

Lubarsch's[23] suggestion that what Maximow observed might have been due to post-mortem changes, is not correct; nor do I think that the case of Lindemann,[24] which he cites, can furnish a confirmation of his view. In this case peculiar crystals, which did not react to methyl-violet, were found in a specimen of a human amyloid liver, which had been preserved in alcohol for some time. It is quite probable that the appearance of these crystals was due to post-mortem changes, which had taken place in the hardening fluid, but they are plainly entirely different from those which Maximow describes in his article.

Granting, then, that in our case the amylaceous bodies were formed by a genuine crystallization around a "nucleus," such as a small lump of ordinary amyloid substance, we should naturally ask the question, whether the same or at least a similar mode of formation holds true in a general way for all amylaceous bodies. I have confined my studies in this regard to the amylaceous bodies of the prostate, since they are most readily obtained. In these investigations I encountered an obstacle which is difficult to overcome at present. The peripheral concentric layers of the prostatic concretions are not only very much thinner than those found in the amylaceous bodies of our case, but their structure seems to be so much more delicate that even very good immersion lenses do not allow the recognition of details with sufficient accuracy. While it is true that one can make out a faint, very dense radial striation in them, it is nevertheless impossible to assure oneself positively that this radial striation is the effect of rows of densely packed needles. However, I think that the coarser radial striation, which is comparatively rare in the prostatic concretions, but which has been described more frequently in the amylaceous bodies of the lungs, also indicates a structure similar to that found in the large amylaceous bodies of our tumor. In the immediate vicinity of the nucleus quite large crystalline needles arranged in radial fashion may be seen more frequently. Posner[25] already has called attention to this fact. He believes them to be

[23] Lubarsch-Ostertag's *Ergebnisse*, Jahrg., iv, 1897, Allg. Path. u. path. Anat., p. 454. Wiesbaden, 1899.

[24] *Centralbl. f. allg. Pathol. u. path. Anat.*, 1897, viii, p. 385.

[25] loc. cit.

crystals of cholesterin, which can hardly be the case, since according to the reports of later authors they are not soluble in ether-alcohol. It seems to me more probable that they are of the same kind as those found in the specimens from the tumors.

A short while ago I compared the crystalline structure observed in the amylaceous bodies of our case with similar crystallizations observed by others in the ordinary amyloid substance. To do this we must, of course, accept the proposition that both substances, the one which is found in the amylaceous bodies and that which composes the amyloid material, are identical or at least closely related to one another chemically. In spite of the statements to the contrary by a large number of competent observers (von Recklinghausen, Ziegler, Posner, Wichmann, Schmidt and others) I am very much inclined to believe that such is the case. In discussing this question, we must not forget that the mere fact that the formation of amylaceous bodies in certain localities has to be regarded as a semiphysiological occurrence, whereas amyloid substance seems to be found under certain diseased conditions only[26] and in other parts of the body, does not in itself constitute a sufficient reason why the material in both cases should not be identical. At least I do not see why in certain localities the production of such a substance should not go on under physiological or at least almost physiological[27] conditions, whereas in others it originates in case of disease only.

Again the differences which they frequently show in their reactions to solutions of iodine, methyl-violet and other chemical reagents (osmic acid, carmine [Posner]) cannot be looked upon as rendering their identity impossible, because these reactions are varying and are not quite constant in either the amyloid substance or the amylaceous bodies. This is a generally recognized fact with the amyla-

[26] Krawkow, *Arch. f. exp. Path. u. Pharm.*, 1898, xl, p. 195. Lately Krawkow claims to have found a material that is at least very similar to, if not identical with, the amyloid substance, in the normal aorta of horses, in the ligamentum nuchæ of cattle, in the stroma of the spleen of calves and the mucous membrane of the stomach of pigs.

[27] Stilling (Virchow's *Archiv*, 1884, xcviii, p. 1) attributes the formation of amylaceous bodies in the prostate to a stagnation of the secretion due to pathological changes in the organ such as hyaline degeneration of the muscle in the neighborhood of the ducts, the *myxangioitis hyalinosa* of von Recklinghausen.

ceous bodies. In the case of the amyloid substance Virchow very early pointed out the remarkable differences in reaction observed, when this material is treated with dilute solutions of iodine and sulphuric acid. The recent observations on the experimental production of amyloid degeneration have demonstrated, that certain forms, apparently the earlier stages, will give a typical reaction with methyl-violet, but do not react with solutions of iodine, showing that even the simple iodine reaction is not a constant phenomenon. Going a step further we may even assert that sometimes in the very earliest stages the body which is deposited in the tissues, does not give either of the two reactions, for I believe with Wichmann that the careful researches of a large number of observers—Litten[28] among the first—leave no room for doubt that the "hyaline" material which is sometimes found with the amyloid substance, or alone in cases in which we might expect to find amyloid, may become transformed in course of time into true amyloid with typical reactions. This assumption is in accordance with the findings in our case, in which lumps and bands of more or less homogeneous material were present, which gave a partial reaction with the methyl-violet; the central parts (probably the older ones) staining purple, the periphery violet. Some of the recent experimental work also seems to be confirmatory of this view. Lubarsch[29] found after seventeen weeks in the spleen of a dog, that had received numerous injections of turpentine, several arteries with typical hyaline degeneration; and four weeks later in another piece of the same spleen hyaline and amyloid arteries were demonstrated. He also found hyaline and amyloid arteries at the same time in the spleen of a rabbit. It must be understood, however, that the "hyaline" substance is not found with the amyloid substance frequently enough to justify the conclusion that this primary formation of "hyaline" is a constant occurrence, but such may be the case and, what seems to deserve some attention, the transformation of one into the other takes place without any perceptible morphological changes in the material. On the other hand we know how comparatively easily

[28] *Deutsche med. Wochenschr.*, 1887, xiii, p. 517.

[29] Lubarsch-Ostertag's *Ergebnisse*, Jahrg., iv, p. 456.

the amyloid substance loses its characteristic reaction. All hardening fluids impair it and tissues which have been hardened in Müller's fluid for some time, give a very imperfect or no reaction at all. Litten[30] showed that amyloid substance when brought into the peritoneal cavity of living animals loses its reaction before being absorbed—a point which has been confirmed by Grigorjeff.[31] In all these cases the macroscopic and microscopic appearances of the substance are not changed in any way.

In view of these facts, I think that we have a right to be a little doubtful whether the presence or absence of the reactions with iodine and methyl-violet indicates a difference in the chemical nature of the body itself, or whether the reactions are not due rather to the admixture of some foreign substance from the outside—a view that, at least in the case of the amylaceous bodies, has been expressed more or less definitely by more than one writer on the subject. But even if there should be a change in the chemical composition, it cannot be a very fundamental one. In this connection, also, we approach the most interesting and much-discussed question whether the staining reactions so commonly employed in the microscopical technique are really all chemical reactions; or if such is not the case, which of them have to be considered as such; but it would lead us too far away from our present subject to enter into a discussion of this point. Even if the change in reaction should indicate some slight chemical alteration, I think it would be better not to over-emphasize the importance of one of these reactions by calling the substance "amyloid," although of course I understand the historical and other reasons which might be adduced in favor of the use of this term. If we should go back to Rokitansky's term "lardaceous[32] substance," we should be able to unite what is now called "amyloid" and the primary "hyaline,"

[30] loc. cit.

[31] Ziegler's *Beiträge*, 1895, xviii, p. 37.

[32] The comparison to lard is not very elegant or striking. It was used by Rokitansky not exclusively for amyloid conditions, but it has been widely adopted by numerous authorities, especially among English writers; nor is it so apt to cause confusion as a new term undoubtedly would. Colloid might be suggested as an alternative, but to my mind it would be best to restrict this term more and more to what P. Ernst has called epithelial colloid (Virchow's *Archiv*, 1892, cxxx, p. 377).

which is now described as an intermediate product in the formation of amyloid substance proper, under one heading and to speak of lardaceous degeneration with or without amyloid reaction—a form of expression, which to my mind has certain advantages to recommend it. In the first place it does not put any stress on the more or less inconstant reactions, and besides would separate the "hyaline" which sometimes precedes and accompanies the amyloid from the heterogeneous substances now comprised under this name. I believe with other authors that only in this way—by trying to eliminate as many substances as possible from this general group—will it be possible to arrive in course of time at a satisfactory definition of what should be called "hyaline."

Coming back after this digression to our original theme, I think that the above considerations show that neither the differences in the conditions under which they are formed nor the differences in reaction encountered constitute a sufficient reason for a separation of the "amyloid" material proper from the substance of which amylaceous bodies are composed. In fact the frequent similarity in reaction, if it proves anything at all, makes it rather probable that the two substances, if not identical, are at any rate closely related chemically, since, although they may not exhibit these reactions from the beginning in one form or another, both of them may do so in the later stages of their formation. Another strong reason in favor of this conception may be found in a fact which has been already sufficiently emphasized by Hildebrand. In his case and also in our own, "amyloid" substance as well as amylaceous bodies were formed in the same locality simultaneously in such intimate local relation with one another, and both presenting such varying degrees of staining reactions, as to make it difficult to believe that the one body could be very different from the other.

But in spite of all this there remains one important *morphological* difference. Amyloid substance appears in irregular lumps and bands, whereas the amylaceous bodies have a characteristic form and structure, possessing a central nucleus of amyloid or other material (pigment, remnants of disintegrated cells) surrounded by regular con-

centric layers. This remarkable regularity in structure has been dwelt upon by many authors and has led some, Lubarsch among them, to compare the process to a crystallization. After what I have seen in my case I am convinced that the process not only may be likened to a crystallization but really is one, adding in this way a new example to the few already known of a crystallization of albuminous substances—a theme, which I have treated a little more fully in a paper read before the Microscopical Society of San Francisco on November 1, 1899.

Amylaceous bodies and amyloid substance differ from one another to this extent: in the one the material is present in amorphous, in the other in crystalline form, although, as is shown in Plate VII, Fig. 8, crystallizations may occur also inside amorphous lardaceous material.

In regard to the mode of formation of the lardaceous substance I have to add very little. It would be entirely beyond the scope of this paper to review the history of the genesis of amyloid substance, more especially so, since in Wichmann's[33] excellent article "Die Amyloiderkrankung" we have a remarkably able and complete review of the subject. From the study of the case described here in detail, and from subsequent examination of thin sections from several cases of amyloid degeneration I can only confirm with Lubarsch Wichmann's statement, that in "amyloid degeneration" we find no conclusive evidence that the amyloid substance is furnished by a degeneration of cells or of intercellular substances, such as connective-tissue fibres or basal membranes. The material is found from the beginning in the spaces between the formed elements; and Wichmann's and Ziegler's hypothesis that it is derived from stagnating albumin seems to be quite acceptable. In the case of the amylaceous bodies the evidence for their formation from degenerating cells seems to be very much stronger, but, in spite of the errors involved in conclusions by analogy, I should suggest it to be more probable that even here the material is not a mere product of degeneration but rather of the nature of an infiltration from the outside. Nor would such an explana-

[33] loc. cit.

tion exclude the possibility that the disintegrating cell-bodies might furnish part of the albumin which is used in the formation of the lardaceous substance.

The general conclusions reached may be formulated as follows:

The substance of the amylaceous bodies is identical with or at least similar to amyloid substance. In the amylaceous bodies this material is present in crystalline form, in the amyloid substance in amorphous form.

It would be advisable to use the word "lardaceous" instead of "amyloid" and perhaps to include under this term the peculiar form of "hyaline" which sometimes precedes and accompanies the amyloid substance.

DESCRIPTION OF PLATES VI AND VII.

PLATE VI.

Fig. 1.—About 400 x. Specimen stained with Van Gieson's method; mounted in xylol-balsam.

Fig. 2.—520 x. Specimen stained with Van Gieson's method; mounted in xylol-balsam.

Fig. 3.—110 x. Specimen stained and mounted as in Figs. 1 and 2.

Fig. 4.—110 x. Teased specimen from tissue hardened in 10% solution of formalin.

PLATE VII.

Fig. 5.—110 x. Specimen stained and mounted as in Fig. 1.

Fig. 6.—285 x. Specimen stained and mounted as in Fig, 1.

Fig. 7.—520 x. Specimen stained in aqueous solution of methyl-violet; mounted in aqueous solution of potassium acetate (1:5).

Fig. 8.—About 650 x. Specimen stained and mounted as in Fig. 7.

All specimens were hardened in 10% solution of formalin.

Outlines of figures were drawn with a Zeiss camera lucida.

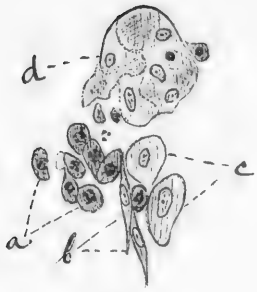


FIG. 1.

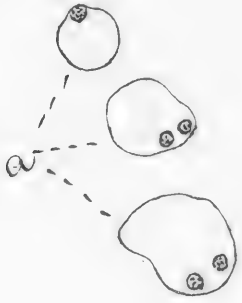


FIG. 2.

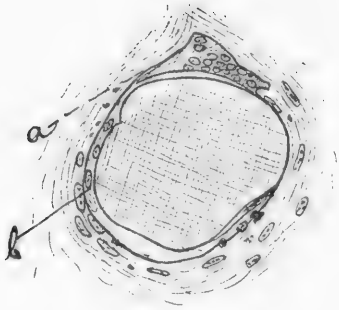
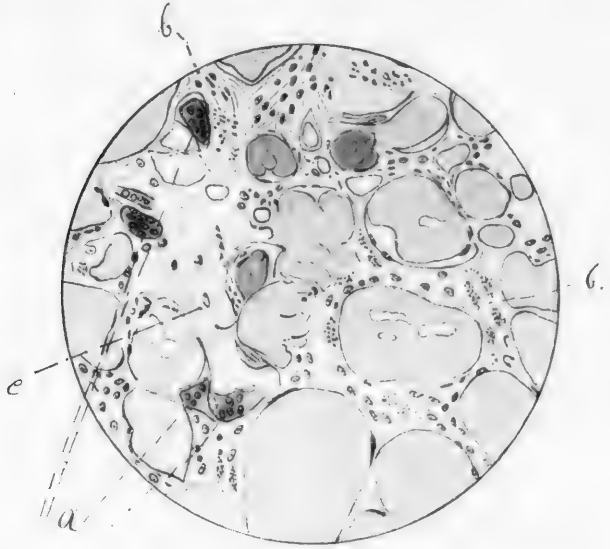


FIG. 3.

FIG. 4.



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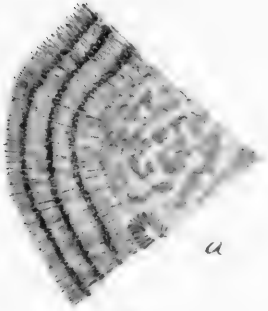

FIG. 7.

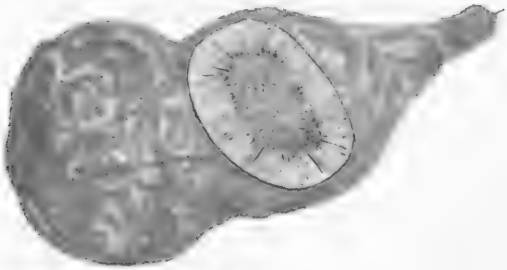

FIG. 8.

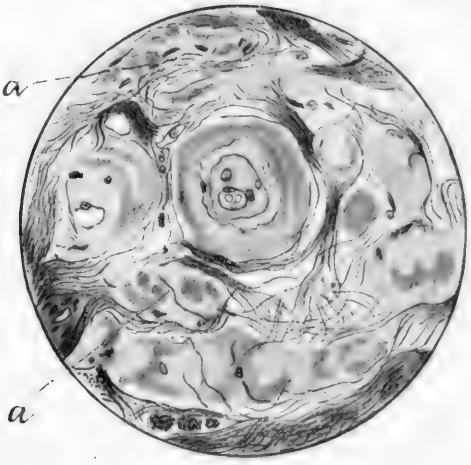

FIG. 6.

ON THE PRESENCE OF NEW ELASTIC FIBRES IN TUMORS.

By ALICE HAMILTON, M. D.

(From the Pathological Laboratory of the Woman's Medical College of Northwestern University, Chicago.)

PLATES VIII AND IX.

The question of the participation of elastic fibres in the hyperplasia of connective tissue has recently aroused a good deal of attention, especially since the discovery of differential stains has made possible the recognition of very delicate elastic fibres. Sclerotic bloodvessels seem to have been first and most extensively studied, with a view to ascertaining not only the fate of the elastic fibres in the media, but also the nature of the newly-formed fibres in the intima. Langhans[1] was the first to declare that this new tissue consisted largely of elastic fibres, and later Baumgarten[2] and Heubner[3] confirmed this view. More recently Dmitrijeff[4] and Jores,[5] working with better staining methods, have described in detail the processes occurring in arteriosclerosis. Though they find minor differences in the syphilitic and non-syphilitic forms, the process in the main is found to be the same in all, namely, a granular disintegration and final absorption of the elastic fibres of the media followed by an extensive proliferation of elastic fibres in the intima. The thickened intima, in fact, consists almost wholly of delicate elastic fibres.

Both Jores and Dmitrijeff consider that the growth of these fibres in the intima is compensatory, to make up for the loss of elasticity in the media, but they differ in opinion as to the rapidity of their for-

[1] Virchow's *Archiv*, 1866, xxxvi, p. 201.
[2] Virchow's *Archiv*, 1878, lxxiii, p. 90.
[3] Die luetische Erkrankungen der Hirnarterien, Leipzig, 1874.
[4] Ziegler's *Beiträge*, 1897, xxii, p. 207.
[5] Ziegler's *Beiträge*, 1898, xxiv, p. 458.

mation. Dmitrijeff finds them always in the older parts of the tissue and thinks they are the result of a slow chronic process, while Jores finds them appearing in the very early stages of endarteritis proliferans. This opinion is confirmed by recent researches of Czyhlarz and Helbing.[6] Jores believes that endarteritis proliferans should not be classed as a chronic inflammatory process, since it differs from such in any other tissue in this one important characteristic, the production of elastic fibres instead of white fibrous connective tissue. The hyperplastic connective tissue which results from chronic inflammation in other tissues, does not, according to his view, ever consist of elastic fibres. This assertion is disproved by the recent investigations of Melnikow-Raswedenkow.[7] He seems to be the first who has systematically studied, by Weigert's staining method, the normal distribution of elastic fibres and the question as to their increase in different pathological processes. His investigations include the liver, spleen, lymphatic glands, heart, adrenal, and testicle. His conclusions are as follows: In hyperplastic connective tissue there is in almost all cases a proliferation of elastic fibres. This proliferation is always compensatory in character, either to make up for a loss of elasticity in the organ, or to increase its normal elasticity. Thus in cirrhosis of the liver the induration and loss of expansibility caused by the new connective tissue are not so great as they would be were the new tissue not composed largely of elastic fibres. In cicatricial contractions, especially in the myocardium, the new tissue contains many elastic fibres to diminish the evil effects of the contraction. In chronic passive congestion when the organ must force on a large volume of venous blood, we find the new tissue very rich in elastic fibres. On the other hand when there is no need for elasticity, the new tissue consists simply of the white fibrous variety, as in the thickened capsule of chronic perisplenitis and in the sclerotic patches in serous membranes. In almost all cases he finds these fibres derived from the adventitia of the blood-vessels; the fibres grow slowly and are very resistant to degenerative changes.

[6] *Centralbl. f. path. Anat.*, 1897, viii, p. 849.
[7] Ziegler's *Beiträge*, 1899, xxvi, p. 546.

The formation of elastic fibres being thus always compensatory, always for a definite purpose, Melnikow-Raswedenkow thinks there is no reason why they should share in the formation of new growths which are entirely harmful in character; and as a matter of fact, he finds that they do not play any part in such growths. He does not enter into the subject in detail, but states that he has formed this conclusion from the study of many cases of benign and malignant neoplasms; and mentions adenofibroma, fibromyoma, sarcoma, carcinoma simplex and scirrhus, and cystoma glandulare. He thinks that the frequent occurrence of degenerative changes in the centre of new growths is due to this lack of elastic fibres in their stroma and the consequent imperfect circulation.

In the discussion following Ziegler's presentation of Melnikow-Raswedenkow's investigations before the Deutsche pathologische Gesellschaft [8] his results met general confirmation, but Hansemann stated that in a single instance (sarcoma of the lung) out of 150 malignant tumors examined he had found increase of elastic fibres, and Schmorl and Orth added that they had each found elastic fibres in a single tumor, the former in a gastric cancer, and the latter in a cancer of the thoracic duct.

I have been unable to find much that is definite on this subject in the writings of other authors[9] It would seem at first sight entirely possible that, inasmuch as all forms of connective tissue are found as elements of tumors, this one variety should also be found, either as a minor part of the growth or forming its chief constituent. Also, considering that the stroma of tumors is formed from the stroma of the organ in which the new growth occurs, it would seem quite possible that when this organ is rich in elastic fibres the newly-

[8] *Verhandl. d. deutschen patholog. Gesellschaft*, 2te Tagung, p. 235, Berlin, 1900.

[9] After this paper was completed and sent to the editor there have appeared the interesting articles of H. U. Williams, "Concerning the new formation of elastic fibres, especially in the stroma of carcinomata" (Contributions to the Science of Medicine dedicated by his pupils to William Henry Welch, p. 291, Baltimore, 1900), and of W. C. White, "The distribution of connective tissue in new growths" (*Bulletin of the Johns Hopkins Hospital*, 1900, xi, p. 185). These articles cannot be considered in this paper.

formed stroma should also contain them, as it contains new blood-vessels.

In investigating this question as to the participation of elastic fibres in the formation of neoplasms I chose the Weigert stain,[10] which gives very excellent results, irrespective of the hardening fluid used. It can be used without counter stain, the blue-black fibres standing out against a light blue or almost colorless background; or, if it is desirable to stain the cells, the counter stain advised by Weigert, lithium-carmine, may be employed. A good contrast is obtained with hæmatoxylin and eosin, but a much more striking one by rapid treatment with Van Gieson's stain and hæmatoxylin. In such sections the cells are stained yellow, nuclei brownish, the white fibrous tissue pink and the elastic fibres blue-black.

As most investigators are agreed that the formation of elastic fibres in hyperplastic connective tissue is a slow process and that the new fibres come from the preexisting ones, I looked for the best results in benign connective-tissue growths occurring in tissues normally rich in such fibres, and therefore selected first for examination fibromata of the subcutaneous tissue. The result in most cases was disappointing. Elastic fibres were present in narrow strands between bundles of white fibres, but in such small numbers that it was impossible to be sure that they were not the preexisting fibres of the subcutaneous tissue which had been pushed apart by the new growth. In only one case were the fibres more numerous, irregularly scattered in thick twisted bundles. This same appearance was found in a myo-fibroma of the uterus. Better results were gained from adeno-fibroma and pericanalicular fibroma of the mammary gland where the proliferation of elastic fibres was often quite extensive. In the normal mammary gland elastic fibres of moderate thickness are found forming bands around the excretory ducts, while a few very delicate ones may be seen in the stroma between the lobules. In the above-named neoplasms these bands around the ducts are often increased in thickness and fibres can be seen running out into the surrounding tissue, while thick bundles of fibres are found in the interacinous stroma

[10] *Centralbl. f. allg. Path. u. path. Anat.*, 1898, ix, p. 289.

as well as in the stroma between the lobules. The elastic tissue of the adventitia of the blood-vessels is increased and sometimes the fibres pass out into the surrounding tissue. In none of these cases, however, did the elastic fibres form a conspicuous element of the tissue, and in some they were very scanty in number. No tumor of this class was found which consisted of elastic fibres exclusively.

Turning then to the question of their presence in the stroma of malignant tumors I chose as the most promising the scirrhous carcinomata. The tumors examined were from the pancreas, mammary gland and liver. The distribution of elastic fibres in the normal pancreas is similar to that in the mammary gland, namely, in slender bands around the excretory ducts, a few delicate fibres in the interlobular tissue, and of course in the walls of the blood-vessels. The liver is much more abundantly supplied with these fibres, which are present in such large numbers in the interlobular connective tissue that they seem to leave little room for any other sort of tissue. Almost all of the scirrhous carcinomata from these organs which were examined for this purpose were found to contain large numbers of elastic fibres far more than had been found in any of the fibromata examined. The fibres in some cases seemed to come from the preexisting ones around the ducts or vessels, but were far more abundant than in normal tissue and passed from these regions to the surrounding stroma. This was especially true of the two cases of scirrhus of the liver, originating in the bile-duct epithelium. Not only the stroma of the new growth but the hyperplastic connective tissue throughout the organ consisted largely of elastic fibres which radiated from the adventitia of the blood-vessels and bile-ducts. In scirrhus of the pancreas the elastic fibres were not so numerous and were found in thick masses scattered irregularly in the stroma of white fibrous tissue. Two cases of scirrhus of the liver and one of the pancreas showed a peculiar arrangement of these fibres. They formed a series of quite well-marked concentric rings around a centre which was made up of carcinomatous cells in nests lying in a stroma quite free from elastic fibres (Plate VIII, Fig. 1). This appearance might be explained by the more rapid growth of this portion of the tumor, which had pushed

aside the elastic fibres of the more fully-developed stroma. Such an arrangement does not show at all in sections stained by the usual methods.

In scirrhus of the mammary gland the elastic fibres were found around the excretory ducts, around the nests of cancer cells and in the interlobular connective tissue. In some, these fibres were delicate, wavy, arranged in regular bands; in others, thick, short, twisted, arranged in heavy masses. Always the appearance was characteristic of elastic fibres—the variable thickness of the fibres, the wavy outline and curled ends. Usually they occurred in scattered groups, but occasionally they were woven in with the stroma quite evenly. In almost every case they formed a conspicuous element in the new growth.

Contrary to expectation, it was in the soft, malignant tumors of epithelial origin, where the connective-tissue stroma was delicate and there was every evidence of rapid development, that the richest growth of elastic fibres was found. In adeno-carcinoma of the uterus (Plate IX, Fig. 3), stomach and mammary gland (Plate VIII, Fig. 2), the stroma in many instances consisted largely of elastic fibres, and very seldom were they altogether wanting. In several cases these fibres were found only in the thicker trabeculæ of the stroma, and in two instances the deeper part only of the tumor contained them, the fibres ceasing gradually as the margin of the growth was approached.

The only forms of sarcoma which promise success in such an examination are fibro-sarcoma and alveolar sarcoma, and good results were obtained in examples of both these groups. A melanotic alveolar sarcoma showed thick, twisted bands of elastic fibres in the stroma; a fibro-sarcoma showed delicate wavy fibres passing between the cells. In neither of these two was there any apparent connection between these fibres and those in the adventitia of the blood-vessels, but in a fibro-sarcoma of the brain this connection could be plainly seen. This tumor, which I have described in this Journal,[11] evidently originated in the outer walls of the blood-vessels and possessed a stroma made up entirely of elastic fibres, which ran in bands, radiating from the ves-

[11] *Journal of Experimental Medicine*, 1899, iv, p. 597.

sels, or were collected in rosettes. In this case all of the fibres were elastic, in the other two the background for these fibres was made up of slender bands of white fibrous tissue (Plate IX, Fig. 4).

It may be objected that the elastic fibres found in these tumors are simply those which were already present in the tissue in which the growth occurs. In answer to this, several arguments may be advanced to prove that there is in the cases described a formation of new fibres, which is sometimes very extensive. In the first place they are found in tumor masses where there is no question that we have to do with a newly-formed stroma, not an infiltration of the original tissues. In the second place they are found in numbers far greater than those normally present in the organ affected. Thirdly, they are found in scattered masses not in connection with vessels or ducts, although normally the organ in question contains elastic fibres only around the excretory ducts and blood-vessels. Thus in the scirrhus of the pancreas represented in Plate VIII, Fig. 1, the elastic fibres are abnormal in quantity and arrangement. Indeed the number found in the tumor often bears no relation to that normally present in the tissue in which the growth occurs. Several specimens of carcinoma of the uterus contained a stroma very rich in such fibres, while two examples of carcinoma of the lung and several of carcinoma in subcutaneous tissue contained none at all. In scirrhus of the liver different parts of the same tumor show different numbers of these fibres and their arrangement is very often, as in the pancreatic and mammary tumors, entirely different from that in the normal organs.

The term "proliferation" as applied to elastic tissue is, of course, rather loosely employed. Strictly speaking "formation" or "deposition" would be more accurate, though an exact term cannot be found until the nature of the process is better understood. The dispute between histologists as to the cellular or intercellular origin of these fibres is still maintained, and the same lack of agreement is found among those who have studied their formation under pathological conditions. Langhans and Dmitrijeff from their studies of arteriosclerosis conclude that the fibres originate in the intercellular matrix, while Heubner and Jores, working upon the same sort of tis-

sue, decide in favor of their cellular origin. It is impossible for me to speak decidedly on this point from my observations of new growths, but it is certainly true in my experience that the tumors containing the largest numbers of elastic fibres are those which possessed a stroma comparatively rich in connective-tissue cells, which would seem to indicate that the cells play an important part in the formation of these fibres.

DESCRIPTION OF PLATES VIII AND IX.

PLATE VIII.

Fig. 1.—Scirrhous carcinoma of the pancreas, showing elastic fibres arranged in rings around a central area. Weigert's stain—counterstained with lithium carmine. Leitz, Obj. 3; Oc. 3.

Fig. 2.—Alveolar carcinoma of the mammary gland. Weigert's stain—counterstained by Van Gieson's method. Leitz, Obj. 6; Oc. 3.

PLATE IX.

Fig. 3.—Alveolar carcinoma of the uterus. Weigert's stain—counterstained with lithium carmine. Leitz, Obj. 6; Oc. 3.

Fig. 4.—Fibro-sarcoma. Weigert's stain—counterstained by Van Gieson's method. Leitz, Obj. 3; Oc. 3.

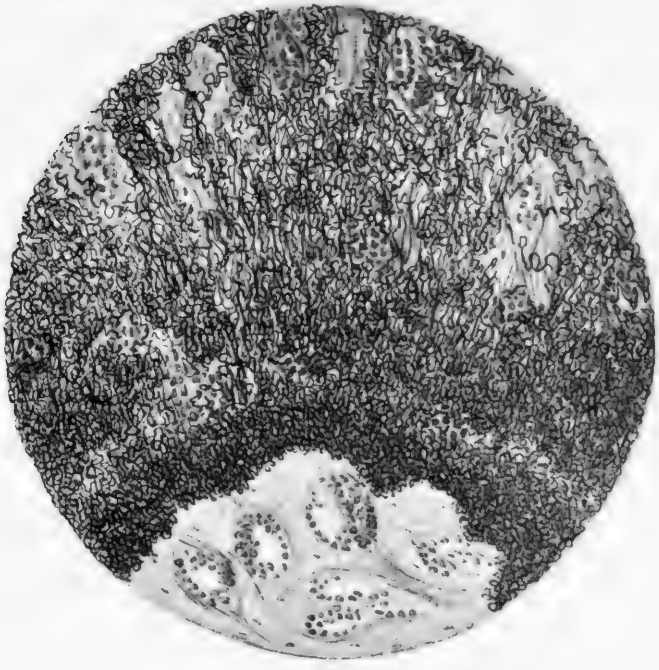

FIG. 1.

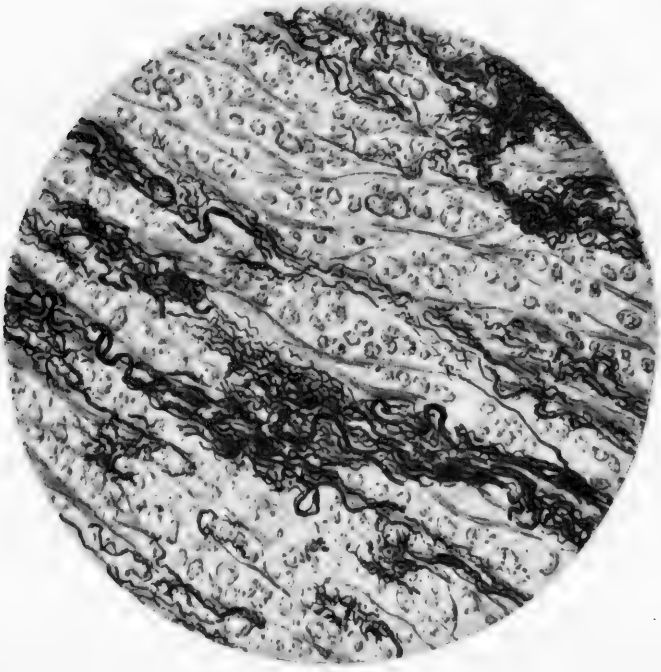

FIG. 2.

FIG 3.

FIG. 4.

A CASE OF GENERAL GASEOUS EMPHYSEMA WITH GAS CYSTS IN THE BRAIN FORMED AFTER DEATH AND DUE TO BACILLUS MUCOSUS CAPSULATUS, WITH A CONSIDERATION OF THE GAS-PRODUCING PROPERTIES OF CERTAIN MEMBERS OF THIS GROUP IN THE CADAVERS OF ANIMALS.

By W. T. HOWARD, Jr., M. D.

(From the Pathological Laboratory of Lakeside Hospital, Cleveland, O.)

Plate X.

My attention was directed to this subject by the following case:

M. D., female, white, aged 40 years, was admitted to Lakeside Hospital, service of Dr. H. S. Upson, December 24, 1898. On admission she was comatose and never regained consciousness. Her pulse was 108 to the minute, regular and rhythmic. Her respirations were of the Cheyne-Stokes type, apnœa lasting twenty seconds. The extremities were cold and cyanotic; the skin was cool and moist. Examination of the chest and abdomen showed nothing abnormal. There was incontinence of urine, and no urine could be obtained on catheterization; hence examination was impossible. Her friends stated that she was in the habit of taking large quantities of morphine. She died eight hours after admission. The clinical diagnosis was morphine poisoning.

No swelling or subcutaneous emphysema of the chest or other part of the body was noticed during life. Immediately after death the body was placed in the refrigerator, kept constantly at 30° F.

Autopsy.—Anatomical Diagnosis: General gaseous emphysema of the subcutaneous tissues, the heart, blood-vessels, liver, spleen and kidneys. Gas cysts of the brain. Septicæmia due to B. mucosus capsulatus (aërogenes group). Fatty degeneration and cloudy swelling of the heart, liver and kidneys. Chronic interstitial nephritis. Ulceration of the stomach and ileum.

The autopsy was begun 24 hours after death. The body was 157 cm. long, very cold; rigor mortis present. The skin over the posterior por-

tions of the body was of a deep purplish hue. The skin and subcutaneous tissues of the face, neck and chest were markedly swollen, and tympanitic on percussion. On section, the skin and subcutaneous tissues were crepitant to the touch and separated from the underlying structures. The subcutaneous and muscular tissues of the chest, neck and face were enormously distended with gas and marked emphysematous crackling was elicited on pressure. The circumference of the chest just above the mammæ was 108 cm. The head was of ordinary size, the scalp thick and firmly adherent. There were no wounds, contusions or abrasions about the face or head. The skull was of ordinary thickness. The dura mater showed no changes. All the sinuses contained dark fluid blood in which were a great number of large and small gas bubbles.

The meningeal vessels, both arteries and veins, contained a great number of gas bubbles. The veins were markedly congested. In the pia-arachnoid there were numerous small gas blebs. The cerebral convolutions were normal. The surfaces of the cerebrum and of the cerebellum showed no changes. There was no exudation, either serous or purulent, upon or in the pia-arachnoid. The structures at the base of the brain appeared normal.

On section the cerebrum was firm (frozen) and moderately congested throughout. The cortex cerebri appeared normal. In the internal capsule and in the lenticular nuclei, there were a large number of nearly round and oval gas cysts, varying from one to twelve millimetres in diameter. Near the centres of both cerebellar lobes, there were similar gas cysts varying from one to four millimetres in diameter. These cysts had perfectly smooth walls. The cerebellum was moderately congested. The pons and medulla both appeared normal on section. The arteries at the base were not thickened and appeared normal.

The spinal vertebræ were normal. Between the dura and the pia-arachnoid were a large number of large and small gas bubbles.

The extremities were free from œdema and emphysema. The abdomen was markedly swollen, measuring 105 cm. in circumference at the level of the umbilicus. The subcutaneous tissue was resonant on percussion, but no crackling was elicited. The peritoneal cavity and the intestines were distended with gas. The sternum, mediastinum and pleuræ were normal. Both lungs were voluminous, and showed small gas blebs under the pleuræ. On section the lungs were markedly congested, but crepitant throughout. Gas bubbles were expressed from the larger pulmonary blood-vessels. The trachea, larynx, and mouth were normal. The pericardium was normal.

The heart weighed 240 grammes. The myocardium was pale and lustreless, and here and there gas cysts of varying size, surrounded by hyperæmic zones, were seen. All the cavities contained dark red fluid blood with gas bubbles. The valves and orifices were normal. The coronary arteries and veins contained small gas bubbles.

The diaphragm was of a pale yellowish hue and felt soapy to the touch, while on section small gas bubbles were seen.

The liver was of ordinary size. The capsule was for the most part smooth. At the under surface of the right lobe, near the gall-bladder, small gas bubbles were found just beneath the peritoneum. On section the liver was rather longer than usual, and had a pale yellowish-gray appearance, with a soapy sensation to the touch. All the visible blood-vessels contained gas bubbles, but the typical appearance of "Schaumleber" was not present. The bile-ducts and gall-bladder were normal. The tissues about the gall-bladder contained small gas bubbles.

The spleen weighed 175 grammes. The capsule contained small gas bubbles. On section the organ was of a dark red color, soapy to the touch, and contained a large number of small gas bubbles.

The two kidneys were of the same size and presented the same general appearances. Together they weighed 325 grammes. The capsules were slightly adherent, the surfaces smooth. The cortices were pale and of ordinary thickness. Both the cortex and the medulla contained small gas cysts. Small gas bubbles were found in the pelves and in the veins. The adrenals and the pancreas were negative. The mucous membrane of the œsophagus was soft and easily removed with the finger. The stomach was of ordinary size. The mucous membrane was markedly congested. Near the cardiac end there were several small ulcers, with indurated bases and edges. Just above the ileo-cæcal valve three Peyer's patches were the seats of small ulcers presenting firm borders. The mucous membrane of the colon and rectum was congested.

The ovaries and tubes were embedded in a mass of adhesions, but presented no changes of special interest.

The bladder, ureters, lymph glands, and the arteries were negative.

The venæ cavæ, the jugular, axillary, portal, hepatic, splenic, renal, and other abdominal veins contained gas bubbles.

Histological Examination of Hardened Sections.—*Central nervous system.*—The veins and capillaries of the pia-arachnoid, the sulci and the cerebral cortex contained great numbers of polymorphic bacilli, which were in some places seen also in the media and adventitia and in small numbers in the surrounding tissue. The bacilli in the vessels were

so numerous that the blood corpuscles were completely displaced. There was no inflammatory reaction. The ganglion cells stained well and there was no evidence of degeneration of the cortical tissue. Plate X, Figure 1 shows a cerebral vessel filled with bacilli.

The veins and capillaries of the pia-arachnoid over the pons, medulla and spinal cord contained great numbers of polymorphic bacilli. In the spinal meninges the vessels were dilated and crowded with bacilli. Some of the vessels were widely distended with gas and a varying number of bacilli were seen near the intima. In some places bacilli were found free in the tissues of the pia-arachnoid.

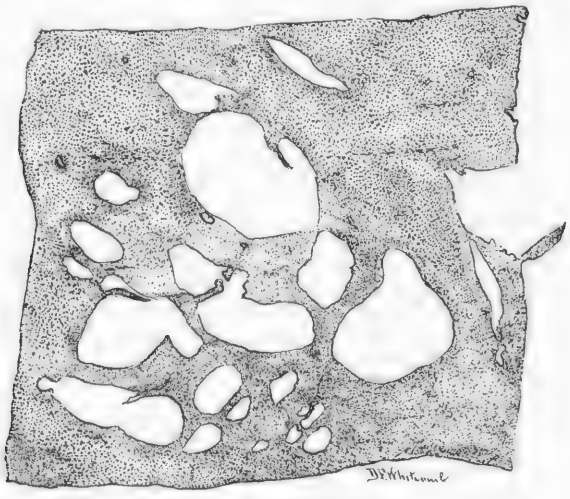

FIG. A.—Gas cysts in the brain.

Sections through the internal capsules and the lenticular nuclei showed large open spaces with regular walls (Fig. A). The tissues were pushed aside by the gas without any necrosis or liquefaction. The cells of the surrounding tissues stained well and appeared normal. There was entire absence of the cell degeneration about the gas cysts, described by Reuling and Herring[1] in their case of gas cysts of the brain. Along the walls of all the gas cysts polymorphic bacilli were seen in varying, and often in great numbers. In the neighborhood of the cysts, the veins and often the small arteries were filled with bacilli. Some of these vessels terminated in the gas cysts. Some, if not all of these cysts had their

[1] *Bulletin of the Johns Hopkins Hospital*, 1899, x, p. 62.

origin in dilatation or rupture of blood-vessels containing bacilli and gas.

In sections through the lateral ventricles the ependyma appeared normal.

The gas cysts in the cerebellum (Fig. B) were identical with those in the cerebrum.

In the internal capsules, lenticular nuclei, and cerebellum many of the veins and capillaries and some of the small arteries were crowded with small polymorphic bacilli. Some of the vessels of the cortex cerebri and of the pons and medulla contained bacilli. The spinal cord was free from gas cysts and its blood-vessels contained no bacilli. No degenerative changes were made out in the brain, pons, medulla, or cord. The ganglion cells were well preserved and stained well.

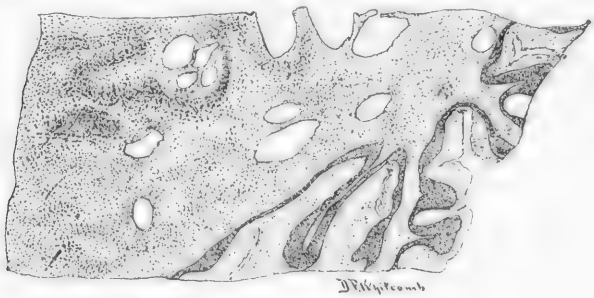

FIG. B.—Gas cysts in the cerebellum.

Heart.—In sections of the heart muscle the nuclei stained faintly and the cytoplasm took the eosin poorly. In some places the striation was indistinct and in others entirely lost. There were areas of marked segmentation of the muscle cells. In a number of small scattered areas the muscle tissue was broken up into a granular amorphous material. Many arteries, veins and capillaries were completely filled with polymorphic bacilli, which were also often found free in the tissues near the blood-vessels.

A large number of small smooth-walled gas cysts were found in the heart muscle. Along the margins of these cysts and in the neighboring tissues bacilli were present in large numbers.

Lungs.—Sections from both lungs showed marked œdema and congestion of the air vesicles, which often contained great numbers of polymorphic bacilli. The blood-vessels were congested, and contained a few bacilli. No gas cysts were seen.

Liver.—The interlobular connective tissue was increased in amount and in many places had encroached very much upon the liver lobules. In many of the interlobular spaces there was round-celled infiltration. There was no congestion of the blood-vessels. Fatty degeneration and cloudy swelling of the liver cells were marked and widespread. In many places the liver cells took the stain poorly, while in others they stained diffusely with eosin. The nuclei could not be made out. Some sections showed a number of gas holes, some of which were evidently due to dilatation of the central veins of lobules. The cysts varied very much in size.

Numerous bacilli were seen in the blood-vessels, especially in the intralobular capillaries and the branches of the hepatic vein. Many capillaries were widely dilated and contained no blood corpuscles but numbers of bacilli.

Spleen.—In the pulp there were few cells that were well preserved. The nuclei did not stain and the outlines of the cells were destroyed. Many cells did not take the eosin stain. The whole tissue had a peculiar amorphous appearance. A few of the Malpighian bodies were preserved. Here and there a few cells containing fat droplets were seen. The red blood corpuscles in the pulp and vessels were poorly preserved. There was no increase of polymorphonuclear leukocytes. No typical gas cysts were found in the spleen. Many of the blood-vessels contained myriads of polymorphic bacilli, the lumina of some being completely filled with them.

Kidneys.—The epithelial cells of the convoluted tubules were swollen and granular. The nuclei of many of the cells did not stain. The glomerular capillaries were congested. There was slight chronic interstitial nephritis, with well-marked chronic passive congestion. The large capsular veins and the arteries, veins and capillaries throughout the kidneys contained great numbers of polymorphic bacilli, the lumina of many of the vessels being completely filled with them. Bacilli were found in large numbers in the tissues, usually in the neighborhood of blood-vessels and within tubules. In many places the tubules were widely distended, forming gas cysts.

Stomach.—The superficial epithelium was lacking in many places. There was marked atrophy of the gastric tubules with round-celled infiltration of the mucosa. In sections through the ulcers only the deeper portions of the glands remained and sometimes both the glands and the muscularis mucosæ had disappeared. The submucosa was greatly thickened by a newly-formed granulation tissue rich in cells and blood-ves-

sels. The cells were fibroblasts, plasma cells and small round cells. A few polymorphonuclear leukocytes, but no eosinophilic cells were seen. No giant cells and no epithelioid cells were found. There was no special infiltration of cells about the blood-vessels and no caseation. Scattered through this granulation tissue, especially in the superficial portions, there were many polymorphic bacilli, quite similar to those found in other organs. No forms so large and thick as Bacillus aërogenes capsulatus were seen here or elsewhere in the organs. In some sections the veins of the submucosa were crowded with bacilli. The muscularis and serosa were normal.

Sections of the *small intestine* showed loss of the superficial epithelium with disintegration of the mucosa in places. In these areas the nuclei and cytoplasm did not stain and the cell outlines were lost. In this material there were numbers of bacilli similar to those found in the organs. The lymphoid tissue showed hyperplasia. In some places in the ileum the submucosa was infiltrated with plasma cells, but there was no granulation-tissue formation similar to that described in the stomach. No caseation and no giant cells were demonstrable. In one place near one of these areas the veins and capillaries were filled with bacilli.

Sections made from the subcutaneous fatty tissue of the neck showed a large number of larger and smaller round or oval gas spaces, the walls of which were lined with bacilli. There were no signs of inflammatory reaction. The tissues about these spaces were pushed aside and compressed. Bacilli were found in large numbers free in the tissue and in the small arteries, veins and capillaries.

Similar changes were found in sections of the pectoral muscles in which gas cysts were numerous. Many of the muscle cells had lost their striation, and in some the nuclei did not stain. Many bacilli were found in the tissues, blood-vessels and gas cysts.

Bacteriological Examination.—Smear preparations made at the time of the autopsy from the emphysematous subcutaneous tissue, the heart's blood, the lungs, liver, spleen, kidneys, cerebrospinal meninges, and brain, all showed great numbers of polymorphic bacilli, which occurred as almost round, oval, and short stout forms with rounded ends, and long thin filaments. They varied from 0.5 to 3 or even 5 μ in length, and were rarely more than 0.3 or 0.4 μ thick. Many were encapsulated. The capsules stained well by Welch's method. No thick bacilli resembling B. aërogenes capsulatus were seen, although careful search was made. Cultures were made from the subcutaneous tissue, the heart's blood, the liver, spleen, kidneys, lungs, brain, cerebral and spinal

meninges, upon blood-serum slants, and in glycerine-agar Petri plates. The cultures from each source, treated both aërobically and anaërobically, were kept in the incubator for twenty-four hours. Anaërobiosis was obtained by the use of Novy's jars.

In all the cultures, both blood-serum slants and Petri plates, the same microörganism grew abundantly and in pure culture. All the Petri plate cultures, both aërobic and anaërobic, showed a large number of round, raised, moist, smooth, opaque, greyish-white colonies. The deep colonies were small, irregular, finely granular, and of a deep brown color, when magnified fifty times. The superficial colonies were large (1 to 2 or 3 mm. in diameter), tended to spread, and microscopically showed irregular, greyish borders, with dark brown centres and a homogeneous appearance.

Cultures and coverslip preparations from all the plates showed the same organism, a polymorphic bacillus similar in all respects to those described in the fresh organs. No large thick bacilli were found in any of the cultures.

In the blood-serum slant cultures the colonies ran together forming a luxuriant spreading growth, in which it was difficult to separate individual colonies. The growth in these tubes was raised, moist, polished and porcelain-like. The water of condensation was cloudy. The growth stuck to the inoculating needle when it was removed from the culture, forming a ropy, mucus-like thread. Careful search failed to disclose any thick bacilli. Morphologically the bacilli in the blood-serum slants were identical with those found in the plate cultures and in the fresh organs.

Further study of this organism, found in pure culture in the subcutaneous tissues, brain, cerebrospinal meninges and other organs, gave the following results: Agar slants after 24 hours in the incubator, or 48 hours at room temperature, showed a luxuriant raised greyish-white, polished, porcelain-like growth, usually with serrated edges. Glycerine and glucose-agar slants gave the same growth. In both these media stab cultures showed abundant gas formation with splitting of the media. Gas bubbles were seen on the surface of the water of condensation, which was cloudy. On coagulated blood serum the growth was like that on agar; but here, in addition to appearing on the water of condensation, gas bubbles were also seen on the growth on the surface of the medium. Liquefaction of the medium did not occur.

On plate- and slant-cultures on gelatine the growth was similar to that on agar, though not so luxuriant. Gelatine was not liquefied. In stab cultures the growth occurred in the form of fine greyish-white colonies

along the track of the needle, with a flattened "nail-head" growth on the surface. Gas production did not occur in plain nutrient gelatine, but appeared in gelatine containing sugars.

On potato there was an abundant growth, best marked on the lower two thirds of the medium, where it was moist and of greyish-brown color. The upper third of the growth was usually dry and granular. The moist lower portion of the growth was porcelain-like and usually spread. Gas formation occurred in the growth, both on the medium and in the water about the potato.

In bouillon the growth was rapid and luxuriant, diffusely cloudy, with a white pellicle covering the surface. At the bottom of the tube there was a copious, greyish-white sediment, which, on shaking, was broken up into a stringy viscid mass.

In bouillon containing sugars abundant gas formation took place.

In Dunham's peptone solution there was a copious growth without indol formation. Blue litmus milk after 24 hours at body temperature had a pink tinge without coagulation. After 48 hours the medium was white with firm coagulation.

The organism was non-motile and stained readily and uniformly with the usual aniline stains. It decolorized slowly when treated by Gram's method. Spore formation did not occur. Capsule formation was sometimes seen in blood-serum cultures, and was constant in the blood and tissue juices of animals dying after inoculation with the bacillus.

Pathogenesis.—White mice died in 12 hours after inoculation with small doses of bouillon, blood-serum, or agar cultures either subcutaneously or intraperitoneally. The bacillus was found at the seat of inoculation, in the blood and in the various organs.

Guinea-pigs were killed within the same time by either subcutaneous or intraperitoneal inoculation. In the peritoneal cavity there was an excess of lymph containing peritoneal cells and leukocytes. Many of these cells enclosed bacilli, a large proportion of which were capsulated.

Rabbits succumbed to small doses (0.25 to 0.5 cc.) of a bouillon culture administered either intravenously or intraperitoneally. Capsulated bacilli were found in the blood and various organs. No gas formation took place in the body during life, but when the animal was kept in a warm place after death, gas was formed.

Rabbits inoculated intravenously with 0.5 cc. of a 24-hour old bouillon culture of the bacillus, killed five minutes afterwards and put in the incubator at body temperature, showed general subcutaneous gaseous emphysema, with gas cavities in the heart, blood-vessels, liver, spleen,

kidneys and free gas in the abdominal cavity with the bacillus in pure culture in the various organs. During the autopsy rabbits were inoculated intravenously with heart's blood, and with blood and tissue juice from the subcutaneous tissues of the chest, and killed. After remaining for 24 hours in a warm place (25 to 30° C.) they were found enormously swollen. At autopsy there was general gaseous emphysema of the subcutaneous tissues and typical "Schaumorgane." Careful examination of coverslip preparations failed to show the long and short thick forms of B. aërogenes capsulatus. There were, instead, great numbers of bacilli morphologically identical with those inoculated. Many capsulated forms were seen.

From the above it is seen that in this case there was septicæmia followed by general gaseous emphysema of the body, the latter developing in all probability after death, caused by an organism belonging to the group B. mucosus capsulatus.

I have compared the bacillus of this case with my B. mucosus capsulatus of hæmorrhagic septicæmia in man[2] and with three other similar bacilli obtained at autopsies during the past winter, and can find no differences, which would seem sufficient to warrant an attempt at the formation of a new class.

This bacillus is practically identical with my bacillus of hæmorrhagic septicæmia except that it gives up the stain more readily when treated by Gram's method. It is an active gas producer in sugar bouillon. When grown in one per cent glucose, saccharose, or lactose bouillon for 48 hours, gas displaces 60% of the medium in the upright arm of the fermentation tube in the case of glucose, 70% with saccharose, and 62% with lactose bouillon. It also produces a large amount of acid, the amount of normal NaOH solution required to neutralize 1 cc. of a 48-hour-old bouillon culture being for glucose bouillon cultures $\frac{1}{13}$ cc., for saccharose or lactose cultures $\frac{1}{25}$ cc.

The tabulated results of a comparative study of the gas and acid production of the bacillus of this case, of similar bacilli obtained at autopsies during the winter, and of my bacillus of hæmorrhagic septicæmia in human beings are given below. For convenience the bacilli

[2] *Journal of Experimental Medicine*, 1899, iv, p. 149.

will be styled No. 34 (the bacillus of the present case), No. 45, No. 46, No. 68 (the numbers of the autopsies from which they were obtained), and H. S. (my bacillus of hæmorrhagic septicæmia in man). Autopsy No. 45 was a case of general arteriosclerosis, with thrombosis of the pulmonary artery and pulmonary infarctions, with B. mucosus capsulatus in the lungs. No. 46 was a case of chronic ulcerative colitis and proctitis with abscess of the left thigh, and a fistulous track communicating with the colon; thrombosis of the pulmonary artery and purulent bronchitis. B. coli communis was obtained in cultures from the colon, the abscess of the thigh, and the heart's blood, liver, and spleen, while B. mucosus capsulatus grew in pure culture from the lungs. Autopsy No. 68 was a case of sarcoma of the right frontal lobe of the cerebrum, and acute croupous pneumonia. B. mucosus capsulatus was obtained in pure culture from the affected lung and the pleura, and also from the uterus. Bacillus H. S. was obtained from the heart's blood and organs of a case of hæmorrhagic septicæmia.[3] In none of these cases, except No. 34 (the present case), was there gaseous emphysema.

Table of Comparative gas Production in Sugar Bouillon.

Bacillus.	1% glucose bouillon.	1% saccharose bouillon.	1% lactose bouillon.	
No. 34	60%	70%	62%	The per cent refers to the ratio of liquid displaced by gas in the upright arm of the fermentation tube.
H.S.	65%	73%	50%	
No. 45	60%	75%	80%	
No. 68	30%	70%	61%	

Table of Comparative Acid Production in Sugar Bouillon.

Bacillus.	1% glucose bouillon.	1% saccharose bouillon.	1% lactose bouillon.	
No. 34	1/13	1/20	1/20	Fractions represent amount, expressed in cc., of normal NaOH solution required to neutralize 1 cc. of bouillon culture.
H. S.	1/13	1/25	1/15	
No. 45	1/30	1/25	1/15	
No. 68	1/25	1/30	1/25	
No. 46	1/30	1/30	1/20	

Strong[4] after an elaborate study of various capsulated bacilli, based more especially upon their gas and acid production in glucose, saccharose and lactose bouillons, divides them into two groups: (1) the Friedländer group, comprising B. pneumoniæ Friedländer, B. ozænæ Fasching, B. sputigenus crassus, Bacillus Wright and Mallory, and

[3] See reference No. 2.

[4] A Study of the Encapsulated Bacilli, *Journal of the Boston Society of the Medical Sciences*, 1899, iii, p. 185.

possibly B. rhinoscleromatis, in which gas-production is most abundant with saccharose, slightly less with glucose, scanty or entirely absent with lactose; slight or no acid formation with lactose; and no coagulation of milk; and (2) the aërogenes group with "more abundant and constant gas formation on all three media; rapid coagulation of milk; and equal amounts of acid formation on all three sugars."

A glance at the tables shows that all four of our bacilli belong to the latter group.

In order to test the relative gas-producing properties of these bacilli in the animal body, rabbits were inoculated intravenously with 48-hour old glucose bouillon cultures of each bacillus. All the rabbits died within 24 hours, without any development of gaseous emphysema during life.

A second group of rabbits were inoculated intravenously, each with 1 cc. of a 24-hour old glucose bouillon culture, which was followed in a few minutes by an intravenous inoculation of 2 cc. of a 20 per cent solution of glucose in sterile distilled water. The rabbits were killed five minutes later and put in the incubator for eight hours, after which time there had developed a slight subcutaneous emphysema, most marked in rabbits inoculated with bacillus 34, and bacillus H. S. The rabbits were all kept sixteen hours longer in a hood at 28° C., when they were found very much swollen. At autopsy they all showed very much the same changes. The subcutaneous tissues of the chest, neck and axillæ were ballooned up and gave distinct emphysematous crackling on pressure. There was usually slight emphysema of the tissues of the abdominal wall and of the thighs. The abdomen was distended with gas. In all the animals the liver was dark greyish-brown in color, soft, and very friable, with emphysematous crackling on pressure, and a peculiar soapy sensation to the touch. The livers were disintegrating. The heart contained dark red blood with a few gas bubbles. Small gas bubbles were sometimes seen in the myocardium. The lungs were distended with gas and well preserved. The spleen showed no special changes. The kidneys showed a few gas cavities, and on pressure gas bubbles escaped from the blood-vessels. In the kidneys of the H. S. rabbit the gas cavities were especially well marked (Plate X, Fig. 2).

A third set of rabbits received intravenous inoculations of 1 cc. of one per cent lactose bouillon cultures of bacilli and a few minutes later 1 cc. of a 10 per cent solution of lactose in sterile distilled water. These

animals were killed five minutes afterwards and treated in the same manner as the second set. At the end of 24 hours they showed subcutaneous gaseous emphysema, and the same visceral changes described for the second set.

The first set of animals (those killed in twenty-four hours by intravenous inoculation of glucose bouillon cultures of the bacilli, but receiving no sugar injection) after remaining in a hood kept at 28° C. for twenty-four hours were very much swollen. The subcutaneous tissues contained considerable gas. Gas was also present in the peritoneal cavity, in the heart and blood-vessels, liver and kidneys. The liver had the same appearances as described for the rabbits inoculated with glucose and lactose. In these animals the gas formation was distinct, but not so abundant as in animals receiving glucose or lactose solutions before death. In several rabbits the stomach wall had ruptured.

In the animals receiving lactose the gas formation was most marked and in these there was a strong odor of putrefaction. The muscles of the chest and thigh were beginning to soften, and on section a thin dark brown juice escaped. Two control rabbits killed at the time of the above experiments and kept under the same conditions for thirty-six hours showed no gas production.

At the autopsies on these animals the bacilli inoculated were recovered in pure culture from the subcutaneous tissues and the heart, lungs, liver, kidneys, in both coverslip preparations and in cultures, both aërobic and anaërobic cultures being made. As a further precaution coverslip preparations were stained by Gram's method. The organisms obtained on the coverslips decolorized completely by Gram, with the exception of the bacillus H. S. By these precautions B. aërogenes capsulatus was excluded as a post-mortem invader in these experiments.

Histological Examination.—Portions of the heart, lungs, liver, spleen, kidneys, muscle, and subcutaneous tissues of the axillæ from each of the various rabbits were hardened in formalin, sectioned and stained with eosin and methylene blue. Very much the same changes were found in the various organs.

The muscle fibres of the heart stained diffusely with eosin; the striæ were lost, and there was segmentation of many of the muscle cells. Only a few nuclei of muscle cells took the blue stain. The cells of the vascular walls usually stained. Large numbers of bacilli were seen in every field of the microscope. They were usually in capillaries and small veins, but were sometimes found free in the tissues. In the sections from the lactose rabbit, a few small gas cysts were found. The lungs

were well preserved and the cells stained well; the blood-vessels and many of the alveoli contained great numbers of bacilli. Some of the alveoli were distended to five or six times their ordinary size and about their margins bacilli were found. The livers showed most marked changes. In most sections the outlines of the liver lobules were entirely lost and the capillary walls had disappeared. There were very few well preserved liver cells in any of the sections. The liver cells were fused together into rows or columns of homogeneous material, in which no nuclei and no cell structure could be made out. These rows and columns were separated by small spaces, corresponding to the capillary spaces. In these large numbers of bacilli were seen. There were no well-defined gas cysts, but the tissue contained a number of irregular spaces. In some sections, especially in those from 46 (lactose), the portal veins were well preserved, and contained red blood cells and bacilli.

The kidneys were fairly well preserved and the nuclei and cell bodies stained well. In all the kidneys, however, in scattered areas, the nuclei of the epithelial cells of the tubules and the cells of the glomeruli refused the stain, while the cytoplasm stained diffusely with eosin and had a granular, coagulated appearance, resembling that of coagulative necrosis. In some places, however, the cells had fused together and presented a homogeneous appearance. In the areas of cellular change the capillaries contained large numbers of bacilli. Most of the veins and nearly all the capillaries contained bacilli in varying numbers. In some sections, as was especially well shown in the kidney of the rabbit inoculated with bacillus H. S. and lactose solution, well marked gas cysts from a pin's point to one or two millimetres in diameter were found. The tissues about these cysts were pushed aside and compressed and large numbers of bacilli were found along their walls. The subcutaneous tissue and muscle collapsed on removal from the body, so that no gas cysts were found in sections made from them. These tissues, however, contained great numbers of bacilli. The bacilli found in the various organs of these animals were apparently of the same species. They varied very much in length, from short oval, almost round forms to threads 6 to 8 μ in length. The long forms were thinner than B. aërogenes capsulatus, and usually had square ends.

Reuling and Herring[5] have reported a case of cavities in the brain produced by B. aërogenes capsulatus. The identification of

[5] *Bulletin of the Johns Hopkins Hospital*, 1899, x, p. 62.

this bacillus in their case depended upon the morphological appearances of the organism in the hardened sections and upon the lesions in the tissues. The cysts were not discovered until after the brain had been hardened in formalin; hence cultures and animal experiments were not made.

In the case of gas cysts with abscess of the brain and cerebrospinal meningitis, and general gaseous emphysema, reported by me,[6] the causal relation of Bacillus aërogenes capsulatus was established by coverslip preparations, cultures, animal experiments and sections of the hardened tissues.

In view of the present case one cannot agree with Reuling and Herring in concluding that B. aërogenes capsulatus was necessarily the cause of the lesions in the two cases of "holes in the brain" with cysts in the heart, lungs, liver, and kidneys, described by Savage and White[7] as cases of "universal cystic degeneration." In the light of our present knowledge, however, it is almost certain that the cysts in these cases were due to the agency of gas-producing bacteria, and probably, on account of its common occurrence in this rôle, to Bacillus aërogenes capsulatus.

SUMMARY.

In the case reported in this article there was septicæmia with special localization of the microorganisms in the brain, with gas cysts of the brain and general gaseous emphysema due to Bacillus mucosus capsulatus (aërogenes group).

The bacillus isolated from the organs of this case, as well as other members of the aërogenic group of Bacillus mucosus capsulatus can cause general gaseous emphysema in the cadavers of rabbits, either with or without the intravenous injection of sugar before the animal is killed, the gas, however, being most abundant and rapidly formed in the former case.

It is not impossible that some of the published cases of gaseous emphysema in which a bacteriological examination was not made,

[6] *Bulletin of the Johns Hopkins Hospital*, 1899, x, p. 66.

[7] *Trans. Path. Soc. London*, 1883, xxxiv, p. 1.

may have been due to members of the Bacillus mucosus capsulatus group.

Dr. Welch has called my attention to the possibility that diabetes may have existed in the case reported in this article. As no examination of the urine could be made, this possibility must be admitted. There is evidence that certain bacteria incapable of producing gas in tissues and organs of the body under other conditions may do so in diabetics on account of the presence of an abundance of sugar.

DESCRIPTION OF PLATE X.

Fig. 1.—Photograph showing B. mucosus capsulatus in a cerebral blood-vessel.

Fig. 2.—Photograph showing gas-cysts and masses of bacteria (B. mucosus capsulatus) in rabbit's kidney.

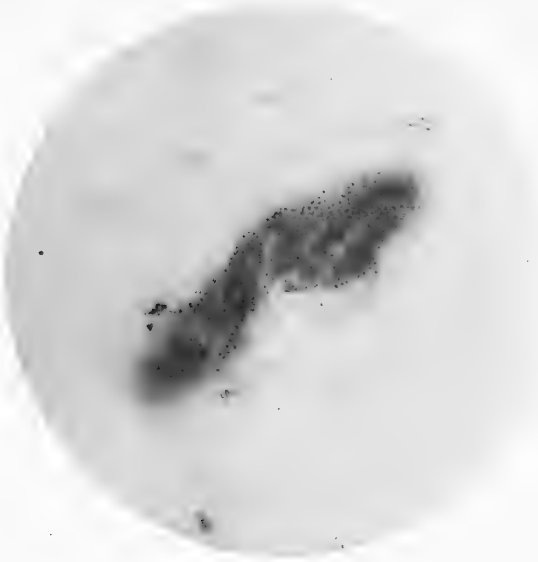

FIG. 1.

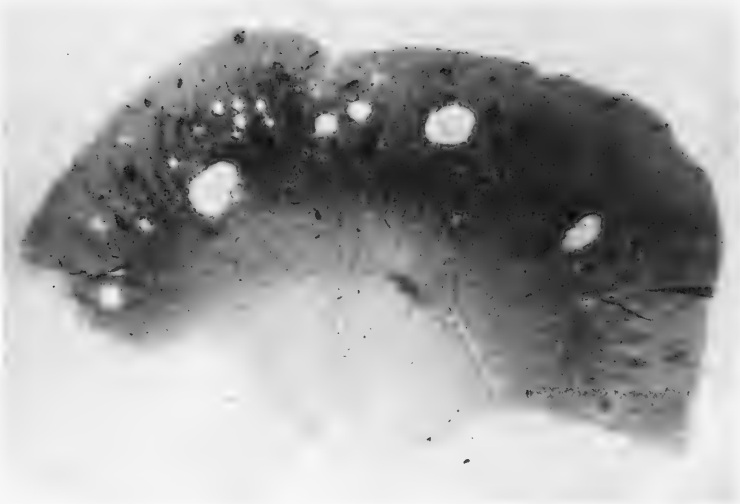

FIG. 2.

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TWO CASES OF NECROTIC BRONCHO-PNEUMONIA WITH STREPTOTHRIX.

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PLATES XI–XVI.

The rarity and importance of such cases as form the basis of this paper, the inadequacy of the study of the similar cases already recorded, and the interesting, though as yet obscure, relationship between an organism or group of organisms which have long been known as actinomyces and the more recently-named streptothricaceæ, have urged us to the somewhat extended study of our material, which it is the purpose of this paper to record.[1]

While the clinical histories are incomplete, the lesions, especially of the lungs, are well defined, and the characters and relationships of the microorganisms present seem to us to be of considerable general as well as special and technical interest.

CASE I. *Clinical History.*—J. L., aged 45, carpenter, was admitted to St. Francis Hospital, December 15, 1898. No history of previous illness. For the past six weeks patient had suffered from cough and moderate dyspnœa. Breath was extremely fetid. Patient was fairly well nourished.

Physical examination on admission revealed a nearly complete consolidation of the right lung, most marked in the lower lobe. Some consolidation of the left lower lobe. The temperature was always high, ranging from 103 to 104° F. The patient did badly and, on account of his continuous coughing and the foul odor of his breath and expectoration, he was isolated.

The clinical diagnosis was gangrene of the lung, following lobar pneumonia. He grew rapidly worse and died eight days after admission.

[1] A brief preliminary report of these cases was made to the New York Pathological Society on March 8, 1899.

Autopsy (Dr. Larkin), two hours after death, December 23, 1898. Body well developed and muscular; no rigor mortis or post-mortem lividity. The abdominal cavity contained a slight amount of straw-colored serum. The left lung was bound down by old adhesions; the lymph nodes at root of lung were very large, measuring from two and a half to three inches in length and one inch in breadth. The mucous membrane of the trachea and bronchi was œdematous, intensely congested, and bestrewn here and there with whitish masses resembling actinomyces "granules." The right lung was bound down by old adhesions and entirely consolidated. Bronchiectatic dilatations were present throughout the lungs. The cut section of the lungs, which were markedly anthracotic, was smooth, glistening, and mostly dark bottle-green in color. The consolidation was firm and resembled that of an organizing pneumonia. The connective tissue septa of the lung were œdematous and grayish in color. Myriads of small yellowish-white foci were scattered through the lungs. The odor of the lungs was extremely fetid.

The kidneys, liver, spleen, pancreas, intestine, bladder, and prostate appeared normal.

Histological Examination.—The microscopic study embraced only the lungs, which were preserved in alcohol. The sections of the various lobes exhibit striking similarity in their microscopic appearances. The anthracosis is not so extensive as we were led to expect from the color and gross appearances of the lungs. The pigment is present in moderate amount in the peribronchial and pleural tissues.

Bronchi.—In places there is a simple catarrhal bronchitis with desquamation of the epithelium, fibrin and leukocytes. The coats of the bronchi are congested and infiltrated with serum and pus cells. The surrounding alveoli are filled with pus cells and a little fibrin. The simple catarrhal bronchitis and peribronchitis are confined to a few tubes. Besides the filling up of the terminal bronchioles and alveoli with leukocytes, a few small miliary abscesses or groups of alveoli filled with leukocytes are found. In the exudate of such alveoli and bronchi only a few streptococci and filaments or rods are present.

Many of the bronchi contain the filamentous colonies already mentioned as suggesting actinomyces granules at the autopsy. Where these are present the bronchi are the seat of a severe and destructive lesion, a necrotic suppurative bronchitis with dilatations, and here the bronchial structure is represented by a tissue staining deeply with eosin, composed of cells with ill-defined outlines and with fragmented nuclei. The

intimate relation of the bacterial or filamentous colonies to the necrosis is clearly seen in the places where the necrosis is strictly limited to the site of attachment of the colonies, although usually the whole circumference of the bronchus is implicated (Plate XVI). The necrosis may affect only the mucosa, or extend through the bronchial coats and about the bronchial dilatations, which contain the largest bacterial colonies, also to the adjacent, compressed and distorted zone of alveoli, which are filled with a coarse network of fibrin staining deeply with eosin. The leukocytes in the lumen of the bronchi near these colonies are in all stages of necrosis and degeneration, and the large amount of detritus found in the tubes is composed mainly of broken-down pus cells.

Pulmonary parenchyma.—The broncho-pneumonic lesion presents two phases of inflammation, which vary in importance and extent in different places. There are, first, zones of peribronchial alveoli filled with leukocytes in a marked condition of nuclear fragmentation, and with fibrin and epithelial cells. The alveolar epithelium in such regions shows little tendency to proliferation, and the alveolar walls are thickened by capillary congestion, by a cellular exudate composed of leukocytes and by epithelioid cells or fibroblasts. Adjacent to or surrounding such alveoli, are second areas of inter- and intra-alveolar pneumonia. Here the alveolar walls are much thickened by a proliferation of polyhedral or epithelioid cells and the fibrin in the alveoli is in all stages of organization. Vascularized plugs of connective tissue obliterate the alveoli here and there. In some sections the areas of interstitial pneumonia are quite extensive. These areas may directly touch upon bronchi which are the seat of an intense necrotic inflammation. Nuclear fragmentation and slight necrosis of the new tissue are observed even at some distance from the bronchus.

The adventitial coats of the arteries, and the connective tissue surrounding the larger bronchi and vessels are much thickened by a serous exudate. In places the necrosis has extended to the walls of the blood-vessels, and the alveoli are filled with red corpuscles. In some sections the hæmorrhagic areas are quite extensive.

Besides the extreme congestion of the capillaries and blood-vessels, few vascular changes exist. Some of the smaller arteries present an obliterating endarteritis. In the larger vessels considerable fibrin and a homogeneous material resembling coral-thrombi are seen. Cocci and filaments are not found in the blood-vessels. The lymph spaces of the bronchi and interlobular septa contain a few streptococci.

The pleura is covered in places by a layer of fibrin and leukocytes,

and is congested, thickened and infiltrated by a serous exudate and by a growth of new tissue. Streptococci are present in the fibrin and in the pleural lymph spaces. Extending from the pleura the interlobular septa and the adjacent alveolar walls are similarly thickened, and the alveoli which have become squeezed and distorted in the areas of interstitial pneumonia thus formed show reversion of the epithelial cells to the embryonal type. The alveoli beneath the pleura are emphysematous, and their epithelium cuboidal.

There is a moderate emphysema throughout the non-consolidated areas of the lungs, the alveoli being filled with swollen and fatty epithelial cells, with but few leukocytes, or the alveoli contain a granular material resembling the alcohol precipitate of albuminous fluid. In places the material derived from the broken-down epithelium stains deeply with eosin. The emphysema is considered to be secondary to the obliteration of the bronchi by the bacterial colonies and exudate.

A few small areas composed of cells which have coalesced or become ill-defined, with small round or fragmented, elongated nuclei, are seen in the sections. Such foci resemble the areas of focal necrosis seen in infectious diseases.

Bronchial lymph nodes.—There is a moderate degree of anthracosis. The congestion of the blood-vessels and the capillaries is intense, and there is considerable exudation into the trabeculæ and capsules of the nodes. The centres of reproduction are enlarged, and the proliferation, especially of the small, round, lymphoid cells, is marked. The lymph sinuses are compressed and show but little desquamation of their lining endothelium. No streptococci or other bacteria are demonstrable in the lymph nodes.

In general the lesions consist of an acute exudative and necrotic inflammation of the bronchi and surrounding alveoli; a broncho-pneumonia, associated with a productive inflammation of the framework of the lungs and organization of the alveolar exudate—an interalveolar and intraalveolar pneumonia. Apart from these lesions, there is an interstitial inflammation and thickening of the pleura and interlobular septa, which perhaps is more chronic in character.

Case II. *Clinical History.*—F. F., aged 23, a laborer, entered Roosevelt Hospital, in the service of Dr. Delafield, January 13, 1899. His mother died of pneumonia. He was a moderate drinker. No syphilis or rheumatism. He has had measles, scarlet fever, diphtheria, and

Pott's disease of spine (?). He has had a chronic cough, since his tenth year, with muco-purulent expectoration and night sweats for several years (?). His present illness began about Thanksgiving day, 1898 (?), when he began to lose flesh and strength, and the pulmonary symptoms became more aggravated and were accompanied by dyspnœa. After Christmas, 1898, he became worse, complaining of headache, anorexia and general malaise followed by chills and a febrile movement, profuse sweating, increase of cough and expectoration, and pain in the chest.

On admission: Moderate emaciation and prostration. There was scoliosis of the dorsal vertebræ. Temp. 101° F.; Pulse, 112; Resp. 28. Examination of urine was negative.

Physical examination: Coarse breathing over left lung in front and behind, with mucous râles. Over lower part of right lung, behind, flatness, cavernous voice and breathing, gurgling and mucous râles. In front, breathing is coarse and high pitched, and there are many coarse râles.

In the hospital he became rapidly worse, with frequent cough and abundant expectoration, his breathing rapid and labored, and death followed pulmonary œdema on January 17, 1899.

Autopsy (Dr. E. Hodenpyl), 24 hours after death: The pleuræ of both lungs are covered in places with a fresh layer of fibrin. Both lungs are largely consolidated, the right lower and the left upper lobes being entirely consolidated. The left lower and the right upper lobes present the lesions of a broncho-pneumonia. The bronchi of both lungs show bronchiectatic dilatations, and numerous grayish actinomyces granules adhere loosely to the congested mucous membrane. On section the surface of the consolidated areas appears smooth and is studded throughout with grayish spots, which correspond to the cut sections of the bronchioles or smallest bronchi.

The bronchial lymph nodes are much enlarged and congested. On section, the nodes appear intensely hyperæmic and studded with small grayish foci.

No noteworthy lesions were observed outside of the respiratory organs.

Histological Examination.—This, as in Case I, was limited to the lungs, which were preserved in alcohol. The lesions in the lungs are strikingly similar to those of Case I.

Bronchi.—Many of the smaller and larger bronchi are the seat of a catarrhal inflammation. The bronchial walls are congested and infiltrated by a serous exudate, with polymorphonuclear leukocytes, and the lumina contain leukocytes, fibrin and granular detritus. The adjacent

peribronchial alveoli are filled with leukocytes and desquamated epithelial cells.

The bronchial "granules" or colonies are composed of a felted network of filaments and rods, identical in appearance with those in Case I.

The bronchi are invariably necrotic where the granules are attached, or the necrosis implicates the whole of the bronchus and its adjacent alveoli. The necrotic inflammation is most marked in the walls of the dilatations, where all traces of the normal bronchial structure have disappeared. The cellular exudate in the bronchi adjacent to these colonies is necrotic, the leukocytes being transformed into a granular mass staining faintly with eosin. The largest bronchiectases are about a third of an inch in diameter, fusiform or sacculated, or irregular in outline. The alveoli around the dilatations are compressed and filled with fibrin almost to the exclusion of leukocytes. The fibrin is less compact and dense than in Case I.

Pulmonary parenchyma—The peribronchitic zones of pneumonia present a variety of appearances and consist of alveoli filled with leukocytes and a little fibrin. The alveolar walls are somewhat thickened by a cellular exudate, composed of cells of the epithelioid type, and the network of fibrin encloses a few epithelioid and polyhedral cells. The organization of the fibrin is in places well advanced.

As in Case I there are extensive areas of interalveolar or interstitial pneumonia. A few giant cells apparently formed by coalescence of the alveolar epithelial cells are found in the distorted alveoli enclosed by the new tissue.

The pleura is covered in places with a layer of fibrin and leukocytes, and is considerably thickened by a new growth of œdematous tissue. The blood-vessels are much congested. The exudate on the surface is more or less organized. Throughout the lymph spaces of the pleura and in the fibrin streptococci are found.

The interlobular septa and the alveolar walls adjacent to the pleura and septa are considerably thickened by a new growth of tissue composed of epithelioid and polyhedral cells and a homogeneous or slightly fibrillated basement substance, and the lining epithelium of the compressed alveoli has undergone reversion to the embryonal type.

The alveoli outside of the areas of broncho-pneumonia are somewhat emphysematous and contain large desquamated epithelial cells, which are markedly fatty or vacuolated.

From an examination of a large number of sections, the impression is gained that with the presence of the streptothrix colonies in the bronchi, the catarrhal inflammation is followed by a necrotic one.

No intra-alveolar hæmorrhage has occurred. The blood-vessels throughout the lungs are greatly congested, and the adventitial coats of the large vessels are markedly thickened and œdematous. No thrombosis was noticed, and the intravascular fibrin formation is moderate.

The *bronchial lymph nodes* show the same condition of acute inflammation as in Case I. Scarcely any pigment and no bacteria are found in the sections.

The lesions here, as in Case I, consist of an acute exudative and necrotic bronchitis, with bronchiectasis, a broncho-pneumonia, composed of alveoli filled with a cellular or fibrinous exudate, which is more or less organized, and a productive inflammation and thickening of the framework of the lungs—alveolar walls, interlobular septa and pleura.

The sections of the lungs of both cases are so similar that the cases can be distinguished from one another only by a comparison of several sections. The exudative phases of the inflammation in the bronchi and broncho-pneumonic areas and the proliferation of the alveolar epithelium in the non-consolidated areas are more marked in Case II than in Case I. The interalveolar and intra-alveolar pneumonia and the changes in the pleura and septa are more advanced in Case I. Case II, therefore, represents an earlier and less advanced stage of a lesion identical in its histological characters with that of Case I.

DESCRIPTION OF THE FILAMENTOUS STREPTOTHRIX COLONIES OR GRANULES AND OF THE BACTERIAL MORPHOLOGY OF THE LUNGS.

The gross appearance of the colonies in the bronchi as seen at the autopsies, the bacteriological examination of the coverslips and of the sections, and the cultural findings of the lungs of both cases may for convenience be considered together, as they were similar.

In the larger bronchi of both cases, either free or lightly attached to the congested mucous membrane, were found numerous opaque whitish and soft masses varying in size from 3 to 5 mm. In the bronchi of smaller caliber and throughout the necrotic and consolidated portions of the lungs smaller yellowish-white particles were scattered.

The colonies consist of a network of filaments, whose terminal portions are often bulbous. No distinct bulbs or end capsules were demonstrable in the smears or sections.

Examination of coverslips made from the colonies, stained by aqueous solution of fuchsin and by Gram, reveals an immense number of rods and filaments, with many cocci.

The cocci, nearly uniform in size, are found single, in pairs, or in short chains. They stain by Gram's method even after long decolorization in alcohol. Capsules could not be demonstrated by Welch's, Friedländer's or Gram's methods, nor by hot carbolic fuchsin.

The rods and filaments.—The various forms reveal a general similarity in morphology, but vary considerably in length, the shorter forms or rods being 6-10 μ in length, and the longer forms or filaments, two or three times as long as the rods. Many of the rods and filaments are more or less curved, have rough or irregular edges and are pointed or bevelled at one or both ends. Very slender and long filaments staining poorly were found, especially in Case I. The forms with rough edges frequently refuse to stain. The rods and filaments stain by Gram's method, but those which show irregularity in contour often decolorize. All the forms are broader than the tubercle bacillus.

The filaments stain faintly but fairly uniformly with aqueous solutions of methylene blue and of fuchsin. The rods stain irregularly and resemble closely those which were finally cultivated. No branching forms were positively recognized. In the coverslips made from other portions of the lungs the rods are much less numerous and do not predominate over the cocci. To contrast the bacterial morphology of the cases, the filaments on the coverslips of Case II stain more irregularly and are more beaded.

The impression gained after an examination of the coverslips of both cases, is that the rod and filamentous forms are merely variations of one microorganism. No tubercle bacilli were found.

THE BACTERIOLOGICAL EXAMINATION OF THE LUNGS OF BOTH CASES.

Sections.—The streptothrix colonies and cocci are best studied in the sections stained by Weigert's method of staining bacteria.

The filaments have no definite arrangement in the colonies. The streptococci are found in groups or short chains intimately mixed with the filaments. The necrotic cellular exudate in the bronchi contains numerous filaments, many of which are faintly stained and easily overlooked in contrast to the well stained.

A few streptococci and isolated filaments are found in the exudate of the catarrhal bronchitis and in the alveoli filled with fragmented leukocytes and organizing exudate. Where the exudate is mainly fibrinous

or composed of proliferated alveolar epithelium and granular material, no microorganisms are found.

The streptothrix filaments with a few streptococci are present in the necrotic bronchial walls and in the adjacent areas of new tissue, which shows nuclear fragmentation.

Streptococci alone are found in the lymph spaces of the bronchi, interlobular septa, and pleura.

With Sterling's gentian violet the filaments stain intensely and are beaded. In sections stained by this method and by carbolic fuchsin, a similar distribution of the streptothrix filaments is observed.[2]

In the sections of the lungs of both cases stained by Gram's or Weigert's method some of the bronchial colonies, or portions of these, were stained a mahogany or reddish-brown color (Plate XIII, A). The peculiar color reaction is confined to the filaments, the cocci invariably staining blue. The coverslips had been preserved for several weeks before the reaction was observed in the sections and the reddish-brown color fades from the sections in several weeks. The reaction is due to the iodine, as it is found in the coverslips and sections stained and examined in Lugol's solution. Although many of the rods on the coverslips did not give the iodine reaction, it was not confined to any one form of rods. The long, thin, and the irregularly edged filaments stain reddish-brown with noticeable frequency.

The substances known to take this color by iodine solutions are glycogen and the erythrodextrines. Some of the yeast fungi found in healthy stools are also said to stain red. A similar reaction has been noted in certain spore-forming butyric-acid bacilli.

Cultures from the lungs.—In both cases cultures were made at once from the colonies in the bronchi and from various portions of the lungs. Numerous pour- and streak-plates of glycerin and ascites-serum agar were made and grown under aërobic and anaërobic conditions. The ascites serum was slightly alkaline to test paper, the reaction of the glycerin agar was 1.5% acid to phenolphthalein. The original and the dilution plates, none of which were overcrowded, developed colonies which on inspection with a low power appeared identical, and coverslips revealed diplococci and streptococci staining by Gram. The rods were seen only when the solid particles carried over onto the original plates were employed as material for the coverslips. Numerous transplantations

[2] In sections stained by Sterling's gentian violet, the areas of interalveolar pneumonia contain ovoid cells resembling mast-cells and fibroblasts with brilliantly red protoplasmic granules smaller than the eosinophilic granulations of leukocytes.

from the particles (composed mainly of rods), on the original pour-plates, upon fresh media developed streptococcus colonies.

Thus on the ordinary culture media, glycerin and ascites-serum agar and also on Loeffler's blood serum, the streptothrix filaments failed to grow, while from both cases a similar streptococcus with the following cultural characteristics was alone isolated: Broth becomes turbid with a moderate whitish deposit resembling a pneumococcus broth culture more closely than a short-chained streptococcus. On potato no growth at 37° C. In gelatin stabs isolated colonies develop slowly along the puncture. Smears made from the broth culture reveal short-chained streptococci and diplococci. Subcutaneous inoculation of the broth streptococcus cultures and of the pus and colonies of the lungs of both cases in mice were negative.

INOCULATION OF ANIMALS WITH THE STREPTOTHRIX COLONIES AND PUS FROM CASES I AND II.

CASE I.—Three rabbits were injected intratracheally with a broth suspension of the colonies and pus. Two of the rabbits were killed two weeks later, with negative post-mortem results.

Rabbit, No. 3805.—This, the third rabbit, was injected with a small syringeful of the suspension on November 11, 1898, and died February 25, 1899, 63 days after inoculation. The animal is moderately emaciated. Autopsy reveals an extensive empyema of the left pleural cavity, the lung being compressed against the spinal column. The pus has a strong odor, and contains small whitish granules. Coverslips reveal cocci and numerous shorter and longer filaments, similar in morphology to those injected. Glycerin-agar pour-plates planted with the pus of the empyema, yield pure cultures of streptococcus. The mediastinal lymph nodes are enlarged, whitish and cheesy in appearance. Throughout the right lung, numerous whitish foci are scattered; the pleura appears normal. The other viscera are congested. The lungs were distended and preserved in alcohol.

Sections of the *right lung* show a general catarrhal and suppurative bronchitis and large peribronchitic zones of pneumonia. The exudate in the bronchi and alveoli is composed almost exclusively of leukocytes, and contains numerous streptothrix clumps. In the hæmatoxylin and eosin sections the clumps stain deeply with eosin, a few threads or filaments towards the centre being stained by hæmatoxylin. Stained by Weigert's or Gram's method, and by carbol fuchsin (Weigert), the peripheral filaments are seen to possess terminal swellings, and are radially

arranged, converging towards the centre of the clump. The rather dense zone of leukocytes attracted by chemotaxis around the clumps shows marked nuclear fragmentation. No streptococci or clusters of well-defined filaments are present in the lung. The clumps are smaller and do not resemble the streptothrix colonies seen in the bronchi of the human lungs, nor were such clumps seen in these lungs.

The pleura of the *left lung* is covered with a thick layer of necrotic exudate, containing small clusters, streptothrix filaments and streptococci. The pleura is much thickened by new tissue which extends into the adjacent and compressed lung tissue. A large cavity in the upper lobe is filled with a necrotic exudate. The wall of the cavity is composed of rapidly proliferating granulation tissue and of a fibrous zone of well-formed but œdematous connective tissue. The lung tissue around the cavity is compressed, and the alveolar epithelium shows marked reversion to the embryonal type.

There is a diffuse broncho-pneumonic consolidation, mainly of leukocytes, which exhibit marked nuclear fragmentation. There is a general suppurative bronchitis. Numerous streptothrix clumps are present in the exudate. Streptococci and groups of well-stained filaments are less frequently seen. The alveolar walls outside of the areas of consolidation are thickened by a cellular exudate, composed of polymorphonuclear leukocytes and many polyhedral cells or fibroblasts.

The intima of the larger and smaller blood-vessels is swollen, and there is considerable proliferation of its cells. The endothelium is well preserved. The muscular coats of the arteries have undergone hypertrophy, and the adventitia is thickened and œdematous.

The liver sections show a marked round-cell infiltration in Glisson's capsule. There is parenchymatous degeneration of the kidney and congestion of the spleen.

With the empyemal pus a second rabbit was injected intraperitoneally, February 25, 1899. Two weeks later it seemed ill and was emaciated; as death did not intervene, and the animal was recovering, it was killed April 6, forty days after inoculation. The autopsy showed a few whitish recent cicatrices on the peritoneum. With this rabbit, our last chance of isolating the streptothrix of the first case was lost, since as above stated, we had failed to secure a growth on artificial media.

Case II.—Two guinea-pigs were injected in the peritoneum and two rabbits, one intravenously, the other through the trachea, on January 17. The bronchial material for all the injections was suspended in 1 cc. of broth.

Guinea-pig No. 3591 died January 24 with a general suppurative peritonitis. Many adhesions between the coils of intestine and retraction of the omentum are present. The mesenteric lymph nodes are enlarged, yellowish in color, the cross-section of the nodes resembling that of cheesy tuberculous nodes. Numerous small streptothrix granules are found free in the exudate or adhering to the serosa, and the examination of coverslips made from the pus reveals numerous cocci, and longer and shorter filaments and thin rods. Glycerin-agar pour-plates yield streptococci alone. The lymph nodes contain streptococci and filaments.

The *second guinea-pig* died January 24. In addition to a suppurative peritonitis, a serofibrinous pericarditis is found. The lungs and pleura appear normal. The viscera show intense congestion.

A rabbit injected intraperitoneally on January 24 with the pus of the first guinea-pig, was killed April 6. The autopsy was negative.

Rabbits.—A large rabbit, as above noted, was injected through the trachea on January 17, 1899, and killed January 30. Autopsy reveals an empyema of the right pleural cavity. The right lung is compressed and whitish in color and covered by fibrin. Examination of coverslips made from the pus show cocci, and filaments resembling in morphology those injected. The lungs are distended and hardened in alcohol. The pericardial layers are adherent. There is marked œdema of the mediastinal tissues, and the lymph nodes are enlarged, whitish and soft.

The left lung is studded with whitish foci. The other viscera are normal, except for coccideal areas in the liver. Sections of the left lung present a general catarrhal bronchitis, with small round-celled peribronchitic infiltration, mostly localized in areas resembling Arnold's lymph nodules. The whitish foci correspond to areas of broncho-pneumonia.

In the atelectatic or compressed portions of the *right lung*, the alveolar walls are thickened by a cellular exudate composed mainly of leukocytes, and by capillary congestion. There are extensive zones of peribronchitic consolidation, the alveoli and bronchi being filled with leukocytes. The leukocytes of the alveolar exudate have undergone nuclear fragmentation and the alveolar walls are in places infiltrated with leukocytes. Streptothrix clumps and a few isolated filaments with numerous streptococci are present in the exudate. The pleura is covered by a necrotic layer of fibrin and leukocytes, containing clusters of streptothrix and numerous streptococci. Beneath the thickened and necrotic pleura the lung tissue is compressed, and deeper a well-marked zone of new tissue has formed.

Sections of the heart and pericardium show a fibrinous and adhesive pericarditis; the fibrin has undergone hyaline metamorphosis and the pericardial layers and adjacent muscular fibres are necrotic, and infiltrated with leukocytes. Here and there in the myocardium beneath the necrotic layer, a well-marked round-celled infiltration exists. Streptococci are present in the exudate.

The blood-vessels throughout the right lung show more or less marked round-celled infiltration of their adventitial coats. The intima is regularly swollen, and in some arteries a well-marked obliterating endarteritis is made out.

Rabbit No. 3727, the second above mentioned, was injected January 17 in the ear vein with bronchial material of Case II and died February 14. Animal is much emaciated. The left pleural cavity is filled with whitish pus of the consistency of cream. The lung is compressed, the upper lobe being adherent to the ribs. The right pleural cavity is partly filled with a more fluid pus. The pleuræ of both lungs are covered with a layer of fibrin and pus. The other viscera of the rabbit are normal.

Coverslips made from the pus reveal cocci and filaments identical in morphology with those injected. Glycerin- and serum-agar planted with the pus yielded pure cultures of streptococcus. Numerous potatoes planted with the pus remain sterile. The organs of a rabbit were planted with the pus: on the kidneys streptothrix colonies developed, which are described in detail below under methods of isolation.

Histological examination.—The pleura is covered with a layer of necrotic fibrin and leukocytes with numerous streptothrix clusters. The superficial part of the thickened pleura is necrotic and infiltrated with leukocytes. The lung tissue adjacent to the pleura is compressed and infiltrated with small round-celled granulation tissue, extending in the lung to a variable depth from the surface and giving rise to areas of interstitial pneumonia. The alveoli enclosed in these areas assume bizarre shapes, and the alveolar epithelium may become columnar. Most of the sections of both lungs contain several larger and smaller abscesses and as no trace of bronchial structure remains even about the small abscesses, we are inclined to consider them metastatic abscesses.

Streptococci and clumps or clusters of ill-defined filaments are seen in the pleural exudate and in the abscesses where they are much less abundant. The walls of the cavities are formed by a layer of necrotic fibrin. Round-celled granulation tissue extends from this zone directly into the adjacent lung.

There is some dilatation of the bronchi, which in general show only

moderate degrees of catarrhal inflammation. Considerable intra-alveolar hæmorrhage has occurred about the abscesses. In some sections, the pneumonic exudate is extensive, and the infiltration of the alveolar walls has reached a moderate degree. The disintegrated tissue near the necrotic exudate abounds in nuclear fragments, and irregularly staining detritus, which in places have been taken up by large vacuolated or fatty cells.

A few circumscribed nodules composed of epithelioid cells, mostly disintegrated and with fragmented nuclei, occur here and there. Giant cells are not seen.

As regards the bacteriological examination of the sections, one is struck by the scarcity of the streptococci, and although the peculiar homogeneous clumps occur in moderate numbers, clusters of well-defined, or isolated filaments are but rarely seen. The clusters of filaments become transformed into the homogeneous eosinophilic clumps, which stain intensely with Weigert's fibrin method or by Gram. Transitional forms between the clusters of well-defined filaments and the homogeneous clumps occur. The most common one is a granular mass barely staining with hæmatoxylin or with anilin gentian violet, in which, however, a few filaments are definitely recognizable. Such transitional forms were found in all the experimental streptothrix lesions.

Besides the changes of the blood-vessels described in the previous rabbit, there is a general and well-marked hypertrophy of the muscular coats of the arteries throughout the lungs. The liver and kidney show acute congestion and slight parenchymatous degeneration.

The empyemal pus of the previous rabbit (3727) was injected into the ear vein of a rabbit (3752) and into the peritoneum of another rabbit (3806).

Rabbit 3752, intravenous injection of 1 cc. of the pus. Animal died 72 hours after injection.

Autopsy.—The peritoneum is intensely congested, and contains straw-colored fluid. The omentum is œdematous. The liver is congested and mottled. Kidney and spleen are congested. Through the pleura numerous small foci are seen. No cultures were made, as the autopsy was held 24 hours after death. The cause of death is considered to have been an infection with the streptococcus, perhaps increased in virulence by passage through the first rabbit.

Histological examination.—The lungs are highly congested. The blood-vessels in places contain the injected material. The emboli are composed of detritus, necrotic fibrin and leukocytes, with streptococci,

and a few homogeneous streptothrix clumps surrounded by a zone of leukocytes. Fibrinous thrombi, in places necrotic and without evidence of organization, occlude the blood-vessels. The coats of the blood-vessels are the seat of a necrotic inflammation extending to the adventitia, which is infiltrated with a serous exudate.

In the neighborhood of the thrombosed blood-vessels the alveoli are filled with fibrin, detritus and leukocytes, forming small areas of lobular pneumonia throughout the lungs. The numerous small white foci found at the autopsy, correspond to groups of several alveoli filled with leukocytes. In the larger areas the disintegration and infiltration with leukocytes of the alveolar walls are more advanced, the consolidation being composed of a mass of leukocytes, shrunken round cells and nuclear fragments, with necrotic fibrin. The liver contains numerous large areas of focal necrosis. The liver and kidney show parenchymatous degeneration, congestion and bacterial capillary emboli. There is an acute hyperplastic splenitis.

Rabbit 3806 was injected into the peritoneum with 1 cc. of the empyemal pus of rabbit 3727, on February 14 and died eleven days later. There is a general suppurative peritonitis, the coils of the intestines being matted together with fibrinous adhesions. The pus contains numerous cocci and filaments. The serosa of the liver, spleen and diaphragm is covered with a thick layer of fibrin. The mesenteric lymph nodes are enlarged, whitish and cheesy on cross section. The spleen is studded with small whitish foci.

Beneath the pleura of the lungs, numerous smaller and larger whitish areas are seen, the pleura itself appearing normal. The upper lobes of both lungs are completely, the lower lobes irregularly, consolidated. The upper left lobe contains a large cavity filled with whitish material and blood (Plate XII, Fig. 6). The bronchi of the right upper lobe are greatly dilated and contain pus, the consolidation of the lobe being whitish in color.

Histological examination.—The pathological process in the lungs presents the same phases of inflammation as in the lungs of the rabbits injected through the trachea, but the topography of the lesions is different, corresponding to their hæmatogenous origin.

The consolidated lobes contain wedge-shaped abscesses and areas of infarction, composed of alveoli more or less filled with leukocytes, fibrin, and a necrotic material, surrounded by a dense zone of leukocytes (Plate XII, Fig. 7). At the apex of the wedges the central blood-vessel is obliterated by a fibrinous thrombus which is necrotic and infil-

trated with leukocytes. The necrotic inflammation extends through the coats of vessels into the surrounding tissues, which are disintegrated and filled with nuclear fragments. A layer of necrotic fibrin surrounds the zone of pus cells, and the adjacent lung tissue is infiltrated with small round cells, an early stage of limitation or demarcation by fibrous tissue.

Numerous smaller abscesses without apparent connection with the blood-vessels are scattered throughout the lung. The upper lobes, are diffusely consolidated, the bronchi are markedly dilated, and the seat of a mild catarrhal bronchitis, with desquamation of the epithelium. In the lower lobes, there is broncho-pneumonia with suppurative bronchitis. The tissue surrounding the largest bronchiectases, is extensively infiltrated with blood.

The coats of the blood-vessels are the seat of an exudative inflammation most marked in the adventitia, and fibrinous thrombi obliterate some of the vessels. There is a general round-celled periarteritis of the smaller vessels. New tissue is found around the bronchial dilatations in the form of areas of interstitial pneumonia. The lungs in other situations are atelectatic and the alveoli contain desquamated cells and granular material.

The serosæ of the liver and spleen are thickened and covered with a layer of fibrin and pus cells. Numerous streptococci, a few clumps and clusters of streptothrix, and isolated filaments which give the reddish iodine reaction are found in the layer of exudate; the liver and kidneys show acute congestion and a moderate degree of parenchymatous degeneration.

The Malpighian bodies of the spleen are enlarged, the blood-vessels are congested, and contain many leukocytes. The endothelial cells of the capillaries show proliferation and degeneration, and a fibrinous exudate is found beneath the capsule.

To summarize briefly, the introduction into the lungs of rabbits through the trachea of the bronchial material of Case I and Case II, consisting of streptococci, streptothrix rods, pus cells, etc., was followed by suppurative and necrotic broncho-pneumonia with bronchiectases, pulmonary abscesses, and extension of the inflammation to the pleura and pericardium with empyema.

Inoculation of the infectious bronchial material of Case II into the ear vein of a rabbit gave rise to pulmonary abscesses, diffuse pneumonic consolidation of the lungs and empyema. The empyemal pus

of this rabbit injected into the peritoneum of another rabbit produced a suppurative peritonitis with metastatic pulmonary abscesses.

In guinea-pigs, inoculation of the bronchial material of Case II into the peritoneum produced suppurative peritonitis.

In the rabbit the pathological process was acute, the leukocytic infiltration and disintegration of tissue led to the formation of abscesses with well-marked limitation of the process by a zone of demarcating connective tissue.

THE METHODS OF ISOLATION, AND THE MORPHOLOGICAL AND BIOLOGICAL CHARACTERS OF THE STREPTOTHRIX OF CASE II.

Methods of isolation.—The usual laboratory media having been found unsuitable for the cultivation of the streptothrix, the method of streaking the fresh organs of rabbits was resorted to. The organs of normal rabbits, immediately after killing, were transferred to Petri dishes, precautions being taken to avoid contamination during removal. The kidneys were halved. The various organs were planted with the empyemal pus of rabbit 3727 and the Petri dishes placed in covered chambers containing sterilized water to prevent drying of the organs. After two days at 37° C., six elevated whitish colonies, the largest 2-3 mm., developed on the kidneys. On the other organs—liver, spleen, heart and muscle—no growth was visible, but streptococci were found on the coverslips, and recovered in pure culture from these organs. Coverslips prepared from the colonies on the kidney showed rods resembling in morphology the bacillus of diphtheria, mixed with moderate numbers of cocci. The average length of the rods approximates that of the tubercle bacillus; they are somewhat broader than this bacillus. The ends are frequently bulbous, less often distinctly wedge-shaped. Some of the rods are frayed out at the end, an appearance suggestive of fragmentation and not common to bacilli. No indication of branching is found, although it was searched for. Stained with methylene blue, the rods appear beaded or striated. The staining reactions and morphology of the growth on kidneys will be more fully described below.

The kidney growth was planted in broth and on the various organs

of a fresh rabbit. The broth tubes yielded pure cultures of the streptococcus. Again on the planted halves of the kidneys, but on none of the other organs, a growth of rods was obtained. Successive transplantations upon fresh kidneys, and also on the other organs, of rabbits were then made at intervals of three days. In no case did the rods grow, as determined by smears, on any organ but the kidney. A photograph (Plate XI, Fig. 5) shows colonies of the streptothrix growing on the rabbit's kidney.

The second generation on kidney was an abundant one, numerous colonies developing along the course of the streaks. Coverslips of the growth revealed the rods, morphologically speaking, in pure culture. Broth, however, planted with the growth again yielded a pure streptococcus culture. The third generation on kidney was still more luxuriant, the growth forming confluent and raised masses, glistening slightly, and resembling in texture the substance of yeast cakes. The later generations on kidneys were moister and diffusely scattered over the surface. The morphology of the third and later generations differed somewhat from that of the first growth, in that the rods were longer and more frayed out at the ends. The wedge-shaped rods were observed only in the early kidney growths.

The striking predilection of the rods for the kidney as a medium of cultivation, and the complete absence of growth on the other organs of the rabbit, is a fact of singular importance. Many observers have recorded observations on the special suitability of various organs for the growth of a variety of microorganisms, but so far as our knowledge extends, no such striking predilection for a particular organ has been noted.

After several generations on kidney had developed we were somewhat perplexed how to proceed further in our study of the rods which were found later to be a species of streptothrix. The streptococcus was still present as revealed by broth cultures in the third successive kidney growth, and the rods still refused to grow in broth, at least in symbiosis with the streptococcus. The growth of the streptothrix on kidney being assured, methods for separating the two microorganisms engaged our attention. Three methods were tried: First, by taking advantage of a

possible difference in the thermal death point of the two microorganisms. The growth on the kidney was suspended in a number of broth tubes which were subjected, some to a temperature of 50° C., others to 56° C. for ten minutes. A number of trials at both temperatures were made. Both microorganisms failed to survive the exposure, transplants failing to produce a growth on broth and on kidney. The second method, prolonged cultivation from kidney to kidney, the transplants being made after three days' growth at 37° C., we were led to believe would succeed, from a comparison of the coverslips taken from the early generations on kidney after several days' growth at 37° C. and later when the kidney growth was kept at room temperature. It was observed that the rods overgrew the streptococcus at incubator temperature, the coccus first becoming visible on the smears when the cultures were kept at room temperature, when the rods, as was determined later, cease to grow below 28-30° C. approximately. The sixth generation on kidney controlled by broth cultures was a pure streptothrix culture. The continuous kidney cultivation was forced upon us, as at first the rods failed to grow in our broth, a fact which did not encourage us to try the next and third method of separation by washing the mixed kidney colonies, which, however, led to success. A well isolated colony was looped, and shaken in successive broth tubes. Five broth tubes and kidneys were finally planted with the washed bits of growth. The kidneys and one of the broth tubes yielded pure cultures of the streptothrix; one tube gave a mixed culture; the others, a pure streptococcus culture. The broth used for all came from the same stock of beef peptone broth, 1.5% acid to phenolphthalein.

The first broth growth was meagre, and with it glycerin and serum agar plates and broth tubes were planted. Again the broth alone showed a growth. Anaërobic stabs in four raw eggs were tried. In one egg several masses composed of a felted clump of mycelium developed but the cultivation in eggs was considered uncertain and was abandoned.

The persistent refusal to grow on any organ but kidney, or on solid media, had suggested the advisability of trying broth and agar in which kidney infusion replaced the usual meat infusion. Broth and 1.5% agar were made both with human and lamb kidneys, with the usual addition of peptone and salt. The reaction was found neutral to test paper or about 0.4 acid to phenolphthalein. In plants of the media with the mixed growth on kidney the streptococcus alone developed.

It was thought possible that, as in the case of the gonococcus, uncoagulated proteids were essential for growth. Chopped rabbits' kidneys were

added to fluid agar at 43° C. planted and poured. The streptothrix failed to grow, however. The following media were also planted, all with negative results: Agar and broth plus sterilized urine; ascitic serum agar, slightly alkaline, with and without glycerin; Loeffler's and coagulated calf's blood serum; prune juice media with peptone and salt of different reactions, alkaline, neutral, and slightly acid; semi-solid mixtures of gelatin and agar, and Hiss's typhoid plating medium.[3]

Broth cultivation of the streptothrix was pursued for two months, transplants being made at intervals of a week, the growth becoming more vigorous and abundant in each successive culture.

Plating media.—From the eighth generation on broth a growth was finally obtained on solid media—the usual peptone agar with addition of 5% glycerin, made to react neutral or 0.5% acid to phenolphthalein.

Separate portions of the agar media with and without addition of 2% glucose were made to react 0.5 alkaline, neutral, and 0.5 to 1% acid to phenolphthalein. As the acidity is decreased, the plating media become softer. To insure accuracy, each set of media was tested after the final sterilization, and two original and one streak plates were planted with large loopfuls of the eighth generation on broth.

In the first and second series of plates those of 0.5 acid reaction furnished the most abundant growth, the other plates containing only several colonies. In a third series the most abundant growth was obtained on the plates of neutral reaction; several colonies now grew on the media of 1.5% reaction.

At the maximum only 50 to 60 colonies developed on the original plates, in marked contrast to the overcrowded pour-plates planted with bacterial cultures.[4]

Description of the streptothrix colonies on neutral or 0.5 acid glycerin agar.—The colonies are visible at the end of 48 hours at 37° C., and attain their maximum development in the course of a few days. The largest are 3 to 4 mm. in diameter. The growth resembles more nearly that of bacteria than the dry growth described as characteristic for many

[3] Hiss, *Journal of Experimental Medicine*, 1897, ii, p. 677.

[4] The following explanation of the scanty growth on solid media is suggested: Though spores were never observed, the sporulation of the streptothrix in the broth culture may be reduced to a minimum, and thus have escaped observation. The colonies may arise only from the germination of the few spores and the subsequent rapid increase in length of their mycelia; whereas the non-spore-bearing streptothrix rods, which are in the majority after the fragmentation caused by the transfer from broth to solid media, stop growing, or grow so slowly that no colonies visible to the naked eye or under a low power develop. However this may be, solid media, barring the kidney, are not suitable or favorable soil for this streptothrix.

of the Streptothrices and allied microorganisms, the tubercle bacillus, etc.

The deep colonies are pin-head or smaller in size, whitish in color and opaque. Spreading colonies are formed out of these deep colonies by extension of the growth into the medium, or on the bottom of the plate. The borders of these so-called disseminated colonies are made up of smaller colonies or groups of rods which lend them a characteristic appearance. The central opaque portions of the colony resemble the non-spreading deep colonies.

Stab cultures on agar (1.5% acid reaction) planted from the colonies now yielded a fairly abundant growth of discoid and isolated colonies along the puncture, the largest ones being 2 to 3 mm. in diameter, whitish in color and sharply defined.

A scanty, whitish growth was now obtained on coagulated ascitic serum containing broth and sugar, and on Loeffler's blood serum. The colonies adhere firmly to these media in contrast to the loose growth on the kidney and on moist glycerin agar. Firm adherence to the surface of media is characteristic for many species of streptothrix, being due to the penetration of the growth into the depth of the media.

The growth in *broth* resembles the granular variety of a streptococcus culture, the small discoid granules adhering firmly to the sides of the tubes, leaving the broth clear. The differences observed between the early and the later broth cultures are due to the more abundant and rapid growth of the streptothrix as cultivation proceeded. In the later cultures a sticky sediment was constantly present after a few days of growth, the granules becoming larger and fluffy with the increase in size and then sinking to the bottom after slight handling of the tubes.

Two varieties of broth culture occur, the one described above with small, hard and adherent discoid granules, or larger fluffy particles; the other the large clumpy variety of broth culture. The clumpy variety is characterized by the formation of a few large clumps at the bottom of the tube which slowly increase in size; the clumps are dull gray in color, firm and gritty. It was observed in a few of the early broth cultures and later in some broth tubes, which were planted with the cheesy material of the lymph nodes, etc., of rabbits that had been injected subcutaneously with pure cultures of the streptothrix. The clumps are seen to form directly from the bits of cheesy material, which slowly increase in size after 8 to 12 days in the incubator at 37° C.

Fermentation tubes.—In litmus 2% grape-sugar broth, no gas forms. The growth develops equally well in both arms of the tube. The color

begins to change 24 to 48 hours after the growth first becomes visible, the production of acid being slow. Discoloration soon occurs and is most marked in the closed arm. Grape sugar does not increase the rapidity of growth in broth. Other sugars were not tried.

Milk cultures.—In litmus milk after 48 hours, the color changes, soon becoming markedly red. Clotting usually occurs on the fourth day or may be delayed to the fifth or seventh day. The clot is soft, finely divided and occupies the whole of the fluid. The purity of the milk cultures was tested by transplants into broth.

In order to determine the cause of the clotting broth cultures were filtered through sterilized filters and added to sterilized milk. No coagulation of the milk occurred after addition of a few drops up to several cc. of the filtered broth culture, either at once or after several days at 37° C. The coagulum is apparently caused by acid production, no soluble enzymes being demonstrable.

Several determinations of the acidity of broth and milk cultures, after 7 to 10 days' growth, were made. Broth was found to have increased in acidity from 1.5% acid to 4.5% and 6% acid to phenolphthalein.

In Dunham's peptone solution of various degrees of reaction no growth occurs, and on plain or glycerinated potatoes, tested and found to be only a few tenths of one per cent acid, no growth occurs, as confirmed by smears.

The absence of growth on potato is not in consonance with the well-recognized suitability of the potato for the cultivation of many Streptothrices. Potato culture is said to favor the formation of the aërial and spore-bearing hyphæ of a number of species of this genus (Streptothrix Eppingeri), and a characteristic musty or mouldy odor accompanies the sporulating stage of growth. Streptothrix Israeli (?) and a few other species fail likewise to grow on potato.

Vitality.—The streptothrix is short lived. The longest period it was found capable of retransplantation from broth to broth was three weeks. Grown on kidney at 37° C. for four days, and then kept for two weeks in a moist chamber at room temperature, when further growth ceases, successful transplantation on fresh kidney becomes uncertain.

Optimum temperature.—It grows best at 37° C. In the summer months when the temperature ranged at times from 24° C. to 29° C., a few tubes of broth and gelatin showed a slight growth on the fifth or sixth day.

In broth, the streptothrix grows equally well in the absence or presence of oxygen, and no change in morphology was observed in the anaërobic growth. The growth on all media is odorless.

Morphology of the streptothrix.—The morphology of the streptothrix is well shown in the photomicrographs (Plate XI). The streptothrix on the coverslips made from the bronchial colonies and pus, and from the lesions and pus of rabbits and guinea-pigs induced by these, presents rod-like or filamentous forms; no branching forms or bulbs, after appropriate staining or in the fresh condition, were identified.

In the growths on the rabbit's kidney, it still retains its rod-like form, but is somewhat shorter, and closely resembles bacilli belonging to the pseudo-diphtheria group. The club-shaped or wedged ends and bulbous central portions of the rods stain intensely with the dyes, and with aqueous solution of methylene-blue marked metachromatism is present. The grouping in closely approximated and parallel lines is frequent. In the later generations on kidney the ends of the rods become poorly defined or taper off gradually, and stain faintly, the first indications of fragmentation (Plate XI, Fig. 1).

The irregularity in staining with methylene blue and aqueous fuchsin solutions gives rise to a beaded appearance. When dilute solutions of dyes slowly penetrate under the cover glass, the bulbous ends and the round refractive swellings stain more intensely than the rest of the protoplasm; in some places the rods remain unstained, or only a portion of the refractive swellings may stain. The bulbous ends stain deeply with Delafield's hæmatoxylin, whereas the rest of the protoplasma is scarcely tinged.

With P. Ernst's stain (methylene blue and Lugol's solution), every rod possesses one or several intensely black, round spots; these are usually single, situated at one extremity or at both ends, or irregularly distributed in the protoplasm, which stains a faint yellow (Plate XI, Fig. 4). These bodies are often smaller in diameter than the breadth of the rod.

No branching forms were discovered in the growth on kidney, either in the coverslip preparations or in the sections of the growth on kidney. Branching was first positively recognized in the early generations in broth (Plate XI, Fig. 4), the fragmentation of the filaments and the branching increasing with the further cultivation in broth.

As shown in the photographs there are striking differences in morphology obtained after different staining methods. Figures 2 and 3 (Plate XI) are of smears made from the same colony on glycerin agar, stained respectively by methylene blue and by Gram. Figure 2 shows the beading of the filaments and large numbers of small rods or coccus-like forms. In Figure 3, from specimens stained by Gram, these features are not prominent, the staining being diffuse, intense, and only a few

short rods are seen. The contrast in the morphology[5] after the two stains is so striking that we believe many have fallen into error when describing the morphology of various streptothrices and have laid too much stress on such coccus-like forms, which have been often mistaken for spores. By careful focussing the connecting links between the colored portions of the rods (stained by methylene blue) can be recognized. Stress is laid on these observations on account of the frequent neglect of most observers to mention the stains employed.

The viscid deposit in the broth tubes seems to be largely intracellular substance, mixed with rods which stain faintly. Curious forms resembling stalks were observed when this material was examined. They varied greatly in size, and their branches form an intricate network, the meshes of which vary in size from delicate filaments up to very broad stalks.

When stained by methylene blue or by P. Ernst's method, the relative position of the darkly-staining spots in the stalks to each other would seem to indicate that stalk formation occurs by the coalescence of the filaments along their length. Sauvageau and Radais, who suggest this explanation, give an excellent description of similar stalk formations in the two species of streptothrix they described.

Our streptothrix in broth and milk cultures exhibits extreme pleomorphism. There are short rods with bulbous extremities, longer rods with marked beading, or with considerable unstained protoplasm. In milk the short rods, and those with bipolar staining are especially common; and long, thin and curved filaments, with or without irregular beading, resemble the filaments of the bronchial colonies.

The short rods with bipolar staining from the milk cultures closely resemble those recovered from the cheesy lesions of rabbits after subcutaneous injection with pure cultures. The scarcity of such forms in the lesions, and the difficulty of recovering the streptothrix in cultures, unless large loopfuls of the cheesy material are transferred, incline us to regard these as degenerative forms.

Morphology of the streptothrix in the experimental lesions.—In the lesions of the guinea-pigs and rabbits produced by the inoculation of the bronchial material of Cases I and II, the streptothrix was present in large numbers in the pus of the pleura and peritoneum together with numerous streptococci. In morphology the rods and filaments resembled closely those composing the streptothrix colonies of Cases I and II.

[5] Bostroem with the Actinomyces bovis and Aoyama with the species of streptothrix which he has lately isolated, have called attention to this contrast in size presented by streptothrix after various staining methods.

In the pulmonary lesions produced by intratracheal introduction of pure cultures into rabbits the streptothrix is found in clumps, which undoubtedly arise from the discoid granules of the broth cultures, and only a long search reveals a few isolated filaments. In the lesions of the skin or lymph nodes after subcutaneous injections a few clusters of streptothrix filaments or rods with bipolar staining may be found.

The clumps stained by hæmatoxylin and eosin are strongly eosinophilic and homogeneous. The fine radiations which extend from the periphery to the centre of the clump when stained by Mallory's[6] method or by carbolic fuchsin (Weigert) appear as long and variously-shaped bulbs or terminal swellings, which extend into the zone of leukocytes surrounding the clump. In every clump a few distinct bulbs enclosing central well-defined mycelial filaments are found.

In contrast to their faint staining by these methods, the bulbs and terminal swellings stain intensely by Weigert's fibrin-stain (Plate XIV). As a few clumps were found in the bronchi and fibrinous pleural exudates of the rabbits injected with the bronchial material of Cases I and II the streptothrix filaments under certain conditions evidently become transformed or agglutinated into clumps, similar to those derived from the granules of the broth cultures. The few finely granular clusters of more or less disintegrated and faintly-staining rods seem to be transitional forms which may later develop into clumps.

The intense staining of the terminal swellings of the mycelial filaments in the clumps when stained by Weigert is well shown in our drawing (Plate XIV). They closely resemble the bulbous forms of the tubercle bacillus as depicted by Babes and Levaditi in their article on the actinomycotic form of the tubercle bacillus and also by Lubarsch and by Schultz in their articles on the bulbous forms of the tubercle bacillus and other bacteria. For a discussion of the nature of the bulbs, etc., which cannot be entered upon here, we refer to Lubarsch.

The morphology of the streptothrix remains the same when grown in collodium sacs within the peritoneal cavity of the rabbit.

The original smears of the bronchial colonies had been kept for several weeks before being stained by P. Ernst's method and then no chromatic particles were seen. To determine whether this was due to prolonged drying of the smears, even after the usual fixing, smears from broth cultures were kept after fixing by heat for several weeks. They were then stained with hæmatoxylin, by Ernst's method, and by

[6] Mallory and Wright, Pathological Technique, p. 282. Method No. 1. Philadelphia, 1897.

methylene blue. The rods invariably stained faintly, and no granules were found. Prolonged drying changes apparently the staining properties of the plasma. Such observations, however, do not aid in determining whether the granules are nuclear or merely reserve depots of material, but perhaps explain their absence from the original smears.

Curious forms characterized by marked tenacity for retaining the stain after decolorization in alcohol were observed in the later generations on broth. When the smears were stained by hot carbolic fuchsin and decolorized in 97% alcohol, followed by washing in water and drying, the intensely staining forms are bulbous, less often bacillary or coccus-like, or branched (Plate XIII, B). With a methylene-blue afterstain the rods stained blue and these questionable forms retain their brilliant red color. When weak solutions of acids are used, the decolorization is prompt and complete.

The morphology of the streptothrix growing on various solid media, Loeffler's and coagulated blood serum, etc., does not differ from that described above.

Growth in the hanging drop.—The streptothrix is non-motile. The branching of the delicate mycelial threads is seen to best advantage in the hanging drop. The irregular swellings and club-shaped ends of the rods, and the round refractive bodies, some apparently free, are also well observed.

The difficulty of watching the growth was increased, as the streptothrix does not grow at room temperature. In broth, acrogenous spore formation was not observed nor was actual fragmentation seen to occur. We were able to watch the actual increase in length of the small buds or projections from the rods and filaments into branches of considerable length. The observations entitle us to consider the branching a real one, and to separate definitely our streptothrix from the group of so-called pleomorphic bacteria. The trend of recent studies limits the usage of this epithet, to describe not a group, but merely a condition of pleomorphism in microorganisms.

The vast number of branching forms seen in smears made from pure cultures also justify us in considering our rods as a species of the genus Streptothrix.

EXPERIMENTAL LESIONS PRODUCED BY INOCULATIONS OF PURE CULTURES OF THE STREPTOTHRIX.

SERIES I.—The eight rabbits of this series were injected on March 27, 1899, either with the pure growth of streptothrix of the 6th generation

on kidney, suspended in broth, or with broth cultures obtained by washing the 3d generation on kidney, as indicated below.

Intraperitoneal injections: Small rabbit No. 1.—Kidney culture. The animal, moderately emaciated, was killed 42 days after inoculation. Whitish patches of thickening of the parietal peritoneum are found. In places the patches are depressed. Autopsy otherwise negative.

Small rabbit No. 2.—Broth culture. Animal emaciated, was killed 42 days later. Whitish patches somewhat more depressed but otherwise similar to those of rabbit No. 1, are found. Microscopic examination shows the patches to be formed of new fibrous tissue. Pea-sized yellowish nodules are found attached to the intestine and mesentery, and coverslips prepared with the dry material reveal a few rods with a faint bipolar stain, but broth tubes planted with a loopful remain sterile. The cæcum contains coccidial ulcers which extend down to the peritoneal coat.

We believe the lesions described to be late or nearly healed stages of lesions produced by the streptothrix. Both animals received small quantities of culture.

Rabbit No. 3.—To compare the lesions following upon combined injections of the streptothrix and the streptococcus, with those produced by the streptothrix alone, a rabbit was injected in the peritoneum with 1 cc. of a (7th day) broth culture of the streptococcus isolated from Case II, together with the broth streptothrix culture. Animal killed 44 days later; no lesions were found.

Intravenous injections: Rabbit No. 4.—Kidney culture of the streptothrix. Animal killed 43 days after inoculation. Autopsy negative.

Rabbit No. 5.—Broth culture. Killed 43 days after inoculation, autopsy likewise negative.

Intratracheal injections: Rabbit No. 6.—Kidney culture. Killed 44 days after inoculation. Autopsy negative.

Rabbit No. 7.—Kidney culture. Killed 49 days after inoculation. Autopsy negative.

Rabbit No. 8.—Broth culture of streptothrix and streptococcus. The possibility that the streptothrix alone introduced into the lungs through the trachea might produce no lesions, led us to inject 1 cc. of a (15th day) broth culture of the streptococcus isolated from Case II, together with a broth streptothrix culture, into the trachea of rabbit No. 8. We conjectured that the streptococcus would predispose the lung to infection with the streptothrix. This rabbit, killed 48 days later, was the only one of the four animals injected into the trachea that presented lesions.

Sections of the lungs reveal many pin-head to small pea-sized masses. The microscopical examination shows these masses to consist of intrabronchial new-formed tissue. The centres of many of these masses contain a homogeneous body, staining deeply with eosin, made up of the discoid granules of the streptothrix broth culture, which we shall refer to as the streptothrix clump. The peculiar morphological features of these clumps, which were present in the lesions of all the animals of the next series, are described under Morphology (p. 179).

The clumps have attracted around them a more or less dense zone of leukocytes, which is surrounded by a layer of necrotic tissue composed of ill-defined cells (staining deeply with eosin) with long and bizarre shaped nuclei or merely nuclear fragments. The fragmented nuclei of these tissue cells are arranged in lines of chemotactic attraction radiating towards the centre of the clump. The peripheral portions of the masses are formed by a spheroidal or polyhedral-celled tissue. When the intrabronchial mass of new tissue is of large size the normal bronchial structure is no longer seen, and the periphery of the mass is encapsulated by tissue of a more fibrous type. The peribronchial or alveolar walls adjacent to the masses are more or less extensively infiltrated by spheroidal cells.

In places areas of new tissue resembling granulation tissue occlude the bronchi and these may be so large that their original connection with bronchi cannot be definitely made out.

Besides the intrabronchial masses enclosing the streptothrix clumps there is a general catarrhal bronchitis and thickening of the bronchial coats by new tissue composed of spheroidal and polyhedral cells with here and there nodular projections of the submucosa into the lumen of the tube covered over by the bronchial epithelium. The lymphoid nodules of the bronchi are hyperplastic.

The first series of inoculations were in the main negative, the sole exception being rabbit No. 8, which alone of the four animals injected into the lungs, received, in addition to the streptothrix, a streptococcus culture.

The lesions in this rabbit consisted of catarrhal bronchitis with diffuse and nodular newly-formed connective tissue—productive bronchitis—hyperplastic lymphoid nodules, and the intrabronchial masses of tissue formed about the streptothrix clumps, or discoid granules of the broth culture. As will be seen from the description of the lesions of the rabbits of Series II, similar and even more marked lesions follow introduction into the lungs of larger quantities of streptothrix granules ob-

tained from broth cultures. The negative results of this series may therefore be attributed to the small quantities of streptothrix granules injected.

SERIES II.—In this series of inoculations larger quantities of streptothrix granules were injected. The granules were broken up as finely as possible so as to minimize their action as foreign bodies.

With the object of predisposing the lungs to infection with the streptothrix, after completion of the operation for tracheotomy, in all but one of the rabbits, a solution of 2 drops of concentrated ammonia in 15 drops of water was injected into the lungs through the trachea, followed 24 hours later by the intratracheal injection of the streptothrix culture. After each injection the small wound was stitched, and in none of the cases did infection of the wound follow. During the intratracheal injections the animals were inclined to the right side.

Rabbit No. 10.—April 3, intratracheal injection of ammonia followed 24 hours later by one-half of the streptothrix granules of a broth culture. Animal killed 37 days later. Pin-head sized foci and larger areas 5 to 6 mm. in diameter, surrounded by an œdematous translucent zone of tissue, are scattered throughout the lobes of both lungs. The lower left lobe is reddish in color and consolidated. Microscopical examination shows catarrhal bronchitis with considerable exudate in the larger tubes. The bronchial, peribronchial and alveolar walls are infiltrated with spheroidal and polyhedral cells. In the mucosa, nodular intrabronchial projections of new-formed tissue are found, which in places have caused a partial or complete obliteration of the lumen of the tube. The tissue resembles œdematous and vascular granulation tissue, and a drawing shows the canalization of the tissue with spaces lined by bronchial epithelium (Plate XV). In this rabbit there are areas of pulmonary atelectasis, and of lobular or broncho-pneumonia, the exudate consisting of leukocytes, epithelial cells, nuclear fragments and a few small giant cells. There is some necrosis of the cellular exudate. The alveolar walls are infiltrated by a few spheroidal or polyhedral cells. The adventitial coats and the intima of the larger blood-vessels are œdematous. Besides these lesions, the smaller bronchi are occluded by intrabronchial masses containing the streptothrix clumps, similar to those described in rabbit No. 8.

The pulmonary lesions in this rabbit, therefore, consist of intrabronchial masses of new tissue, enclosing the streptothrix clumps, of broncho-pneumonia—obliteration of some of the bronchial tubes and areas of lobular pneumonia with atelectasis.

Rabbit No. 11.—April 20, intratracheal injection of the streptothrix granules of a broth culture (15 days' growth), without the preliminary injection of ammonia. Animal killed 20 days after inoculation. About the trachea are found several large yellowish masses surrounded by fibrous tissue, due to escape of the culture through the track of the needle.

Scattered throughout the lungs small pin-head foci (Plate XII, Fig. 8) most numerous posteriorly, are found, similar in their microscopic appearances to the intrabronchial masses around the streptothrix clumps described above in rabbits Nos. 8 and 10. No other lesions are present in the lung.

Rabbit No. 12.—April 4, intratracheal injection of ammonia followed 24 hours later by an intratracheal injection of half of the streptothrix granules of a broth culture (15 days' growth). Slight embarrassment to respiration followed both injections; animal killed 36 days later.

Small (5 to 6 mm.) flat masses surrounded by a translucent zone project beneath the pleura. The posterior portion of the lung, reddish in color, is consolidated by areas of lobular pneumonia. The intrabronchial masses around the streptothrix clumps are larger than those of the preceding rabbits. The largest masses situated beneath the pleura, which is congested, œdematous and thickened, are composed of a large central collection of pus cells and necrotic tissue, with an occasional streptothrix clump and are encapsulated by an extensive zone of well-formed spheroidal or polyhedral-celled tissue, which extends diffusely beneath the pleura into the adjacent lung tissue. A few arteries present an obliterating endarteritis. The lesions, judging from the larger size of the abscesses, are more severe than those of rabbit No. 10. The encapsulation by fibrous tissue testifies to the successful conservative attempt on the part of the lung to limit the process.

Rabbit No. 13.—May 25, intratracheal injection of ammonia followed 24 hours later by injection of large quantities of streptothrix granules obtained from various broth cultures. Twelve days later the animal was killed.

At autopsy there are several large yellowish masses resembling inspissated pus, surrounded by a fibrous capsule in the peritracheal tissue due to escape of the injected fluid. There is a sero-fibrinous mediastinitis. The upper lobe of the right lung contains reddish, collapsed areas, and yellowish-white pea-sized masses project beneath the pleura. White pin-head sized masses project beneath the pleura. White pin-head sized foci are scattered throughout the lobes of the lungs.

The streptothrix was recovered in pure culture from the peritracheal and from one of the pleural masses in the upper lobe.

Microscopically the subpleural masses resemble those of rabbit No. 12. The foci consist of alveoli filled with spheroidal and epithelial cells. The right upper lobe is extensively atelectatic, and the alveoli contain exfoliated epithelium and large multinucleated giant cells. There are also areas of interstitial tissue of the ordinary type.

Rabbit No. 14.—May 25, intratracheal injection of ammonia followed 24 hours later by injection of large quantities of streptothrix granules, obtained from the same broth cultures with which rabbit No. 13 was injected. Animal, much emaciated and with marked dyspnœa, was killed 12 days after inoculation.

The autopsy showed catarrhal bronchitis with much exudate. A large cavity with dense fibrous walls and filled with creamy semi-solid material occupies nearly the whole upper lobe of right lung. Numerous smaller cavities and white foci are scattered throughout the various lobes of both lungs. The pericardium and left lung are adherent to the chest wall and there is sero-purulent pericarditis.

Microscopically the cavities and abscesses are seen to be surrounded by extensive zones of œdematous granulation tissue. The lung tissue between the abscesses is atelectatic or consists of new-formed fibrous tissue. The pus in the cavity and abscesses contains numerous rods and cocci and a few streptothrix clumps. The necrotic walls of the cavity likewise contain cocci and rods. Cultures yield a variety of microorganisms which were not identified.

The lungs resemble those of the rabbits which were injected through the trachea with the streptothrix masses and pus obtained directly from the original human cases. The result in this experiment indicates that cavity formation and severer lesions are produced when there is concurrent infection with cocci. In this case the cocci were either introduced accidentally at the time of injection, or entered later from the air passages.

Rabbit No. 15.—May 12, intratracheal injection of ammonia followed 24 hours later by injection of the streptothrix granules obtained from various broth cultures. Animal killed 10 days later. Near the trachea two yellowish almond-sized lumps encapsulated by radially striated dense fibrous tissue are found. The lumps are due to the escape of the fluid at time of injection through the perforation in the wall of the trachea.

Upper lobe of right lung is dark red in color, consolidated, somewhat collapsed, and does not distend when the lungs are injected. Sev-

eral confluent whitish dime-sized nodules are seen beneath the pleura. Scattered throughout all the lobes a few subpleural pin-head sized whitish nodules are found.

Microscopically the consolidated lobe is seen to be atelectatic. The subpleural nodules are abscesses which contain a few streptothrix clumps and resemble those of rabbit No. 11. The pleura over the abscess is thickened by a tissue rich in spheroidal cells and leukocytes.

Smears made from masses around the trachea reveal a few rods with bipolar staining, and of the two broth tubes planted with large loopfuls of the material, one alone yields a delayed growth of the streptothrix.

The sections of the abscesses show a few streptothrix clumps without isolated rods. Other forms of bacteria were not seen. The remaining lobes contain smaller intrabronchial masses which surround the clumps, and there is a general catarrhal and productive bronchitis with nodular projections into and obliteration of the lumina of the tubes. The adventitia of the small blood-vessel is frequently infiltrated with spheroidal cells. The vascular changes otherwise resemble those described above.

SERIES III.—The following inoculations are for convenience grouped together.

Intraperitoneal injection: Rabbit No. 16.—April 20, injected into the peritoneum numerous granules of a streptothrix broth culture (15 days' growth). Rabbit killed 25 days later. Over site of injection, attached by a pedicle, is a pea-sized mass, hanging freely in the peritoneal cavity. Six small yellowish nodules adhere to the mesentery of the stomach and to the liver. The mesenteric lymph nodes are not affected. A yellowish-white, small, almond-sized mass is attached to the cæcum by fresh adhesions. On section the larger masses are made up of several confluent nodules, each surrounded by dense fibrous tissue. Smears made from the material reveal a few bipolar-staining rods. Small bits of the material were planted in four broth tubes, of which two after two weeks at 37° C. develop the clumpy variety of streptothrix culture.

The yellowish nodules forming the pendulous mass consist of a central collection of fragmented faintly-staining nuclei and necrotic granular material with a few clumps and clusters of streptothrix surrounded by a zone of vascular connective tissue, composed of spheroidal and larger polyhedral cells and fibroblasts, with eosinophilic granulations. The nodules on the serosa of the liver are of similar structure, the parenchyma of the liver being unaffected. Some of the nodules, however, are formed exclusively of vascular granulation tissue, and represent the nearly healed lesions. (Compare the result in this case with that in rabbits Nos. 1 and 2, in which the process of healing is complete, p. 181.)

Subcutaneous injection: Rabbit No. 17.—Feb. 25, injected subcutaneously the granules of an early broth culture. Twenty-four days later a small almond-sized nodule formed near the site of injection was removed. Coverslips revealed no organisms and no cultures were made. The mass on cross section is yellowish in color, resembles inspissated pus and is encapsulated, with a radially striated border of fibrous tissue.

In the sections stained by Weigert and by Sterling's gentian violet, a few clusters of streptothrix are found. The rods are similar to those met in the sections of the human lungs. The central part of the mass consists of a collection of leukocytes and necrotic material surrounded by vascular granulation tissue, which becomes densely fibrous at the periphery.

Intraperitoneal inoculations into guinea-pigs.—Three guinea-pigs were injected with large quantities of streptothrix culture obtained from various sugar-broth tubes on May 13.

The smallest animal became emaciated and died 13 days later. A few pin-head sized whitish nodules were found loosely attached to the mesentery. Smears reveal a few rods with polar staining; the inguinal lymph nodes were slightly swollen. The second animal was killed two weeks after inoculation. Negative autopsy. The third died 18 days after inoculation. Marked emaciation. A few small white nodules in the peritoneum similar to those found in the first guinea-pig. No cultures were made. No conclusions can be drawn from these inoculations in guinea-pigs, as they were not repeated.

Summary of the Experimental Lesions Produced by Inoculations of Pure Cultures of Streptothrix.—The streptothrix is pathogenic to rabbits and guinea-pigs, but death rarely supervenes from its action. In the rabbit subcutaneous injections and the escape of broth cultures into the peritracheal tissues, and intraperitoneal injections in rabbits and guinea-pigs produce local death of tissue and abscesses encapsulated by extensive zones of granulation tissue. In the lesions a few clusters of well-stained filaments or rods may occasionally remain, but individual rods are rarely found. The few clumps which are present in all the lesions represent, we believe, the discoid granules of the broth culture, which have undergone a peculiar transformation.

Introduction of pure cultures into the lungs through the trachea

is followed by similar phases of reaction on the part of the blood-vessels and of the tissue cells. When large quantities of the finely broken streptothrix granules of broth cultures are injected into the lungs, areas of consolidation composed of proliferated epithelial cells and leukocytes are formed, in addition to the intrabronchial masses of new-formed tissue, which encapsulate the clumps or discoid granules of the cultures. The largest intrabronchial masses in their early stages (two weeks after inoculation) resemble abscesses.

RESUMÉ.

The two cases of broncho-pneumonia in man forming the subject of this article were characterized by intense catarrhal and necrotic inflammation of the bronchi and by the presence of numerous streptothrix colonies in the bronchial lumina.

Introduction of the bronchial material of Case I into the trachea of three rabbits induced pulmonary abscesses and empyema of the pleura and pericardium in one of the animals.

Introduction of the bronchial material of Case II into the ear vein of a rabbit, and into the trachea of a second rabbit induced likewise pulmonary abscesses and empyema.

The empyemal pus of these rabbits contained filaments and rods morphologically identical with those composing the streptothrix colonies of the human cases.

From Cases I and II a streptococcus was cultivated on the ordinary media, the streptothrix not being isolated in culture directly from the human organs. By inoculating the fresh and sterile kidneys removed from a normal rabbit with the empyemal pus of a rabbit injected into the ear veins with the bronchial material of Case II the streptothrix from this case was finally isolated in pure culture, and its morphological and biological characters studied in detail.

COMPARISON WITH SPECIES PREVIOUSLY REPORTED.—Space does not permit a review of the records of the numerous species of the genus Streptothrix which have been found to bear a causal relation to various lesions or diseases, nor can we dwell upon the interesting rela-

tionships of the Streptothrices to a number of microorganisms which heretofore have been considered bacilli or fissure fungi.

For many years Actinomyces bovis Harz (Streptothrix actinomyces Rossi Doria) was considered to be the only representative of a genus of microorganisms to which the name "streptothrix" is at present generally applied.[1] The growth flourished best under aërobic conditions and consisted of a felted network of branching mycelia mixed with shorter coccus- or spore-like forms. Inoculations of the cultures in animals were regularly negative.

Wolff and Israel in 1889 cultivated from two typical cases of actinomycosis a different species, Streptothrix Israeli, which flourished best under anaërobic conditions. In contrast to the morphology of the aërobic species the growth consisted mainly of rods with a few branching filaments, except in egg cultures where branching masses of mycelia developed. It grew only at incubator temperature, whereas the aërobic species developed equally well at room temperature. Typical actinomycotic lesions with rosettes and terminal bulbs followed introduction of the cultures into the peritoneum of rabbits.

Wolff and Israel's observations were not confirmed until A. Aschoff in 1895 cultivated a species similar to Streptothrix Israeli from a case of pulmonary actinomycosis.

Recently E. Levy has reported five cases of human actinomycosis from which he cultivated a strictly anaërobic species, which closely resembled Streptothrix Israeli.

Actinomyces bovis and Streptothrix Israeli are now considered to be two distinct species of streptothrix. The failure of Lucas, working in Levy's laboratory, to transform the aërobic species (Streptothrix actinomyces) into the anaërobic variety, with the morphological characteristics of Streptothrix Israeli, by cultivation through many generations under anaërobic conditions of growth, speaks against the identity of the two microorganisms. Levy does not favor the view held by some that Streptothrix Israeli is a pleomorphic bacillus.

[1] Inasmuch as the name "streptothrix" had been previously appropriated for one of the hyphomycetes by Corda in 1839, it has been proposed by Levy to designate the group now generally called "streptothrix" as "actinomyces." We have, however, followed in this article the more usual designation at present.

Hugo Bruns has isolated what he considers to be a transitional form between the two species. In its cultural and morphological characteristics it closely resembles Streptothrix Israeli, but differs from it, in that it grows best aërobically.

The species isolated by us resembles closely in its morphological and cultural characteristics Streptothrix Israeli; but it is decidedly less pathogenic to animals. We do not consider the anaërobic preferences of Streptothrix Israeli sufficiently marked to form a point of differentiation between it and the species isolated by us, or by Bruns, since Wolff and Israel mention successful primary cultivation of their streptothrix from their first case on slant agar.

On account of the resemblance of Bruns's agar cultures to those of the tubercle bacillus it cannot be identified with ours, and the same objection presents itself in regard to the streptothrix isolated by Foulerton which he considers resembles the species isolated by Bruns.

Berestneff has isolated five species of streptothrix from human cases and although one of his species resembles ours in regard to its morphology and its broth cultures, we do not consider his descriptions sufficiently complete for purposes of species identification.

Paul Kruse cultivated from the yellowish granules in the pus of a maxillary tumor in a diabetic barber, a streptothrix which he considers closely related to or identical with Streptothrix Israeli. Except for the formation of acid in milk and broth cultures our species does not differ from Kruse's as far as one can judge from his descriptions.

Our streptothrix thus would seem to be, if not identical with, at least closely related to Streptothrix Israeli, and to the species isolated by Kruse. Whether it can be considered a variety or transitional form of Levy's anaërobic species must be left undecided.

The pulmonary lesions of the cases, however, do not resemble the broncho-pneumonic type of pulmonary actinomycosis described in the literature. The intense necrotic inflammation of the bronchi of the two cases reported, distinguishes them from the heretofore recorded cases of pulmonary actinomycosis or pseudo-actinomycosis.

We desire to express our thanks to Dr. A. Seibert and to Professor Delafield for the privilege of reporting these cases, and to Dr. Eugene Hodenpyl, Pathologist to the Roosevelt Hospital, for the opportunity of studying Case II.

It gives us great pleasure to acknowledge our deep sense of obligation to Prof. T. Mitchell Prudden for the kind assistance and suggestions which have always been at our disposal.

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DESCRIPTION OF PLATES XI-XVI.

PLATE XI.

Fig. 1.—Rod-like forms of streptothrix of Case II, from 2 days' growth of 3d generation on rabbit's kidney. Gram's stain.

Fig. 2.—Smear from colony, 7 days' growth, on glycerin agar, stained with methylene blue. Shows beaded filaments, small rods, and coccus-like forms. Compare with Fig. 3.

Fig. 3.—Smear from same colony as Fig. 2, stained by Gram. Note the differences in morphological appearances brought out by the two stains used for Fig. 2 and Fig. 3.

Fig. 4.—Coverslip from 3 days' growth in sugar broth. P. Ernst's stain. Shows branching forms and deeply stained granules.

Fig. 5.—Photograph showing streptothrix colonies growing on rabbit's kidney.

PLATE XII.

Fig. 6.—Photograph of section of rabbit's lung (Rabbit 3806) showing pneumonia and cavity produced by streptothrix infection following intraperitoneal inoculation of empyemal pus of infected rabbit.

Fig. 7.—Areas of infarction and abscesses in lung of the same rabbit (Rabbit 3806).

Fig. 8.—Scattered foci of consolidation in lung of rabbit killed 20 days after intratracheal inoculation with broth culture of streptothrix (Rabbit No. 11). With higher power these foci show intrabronchial masses of connective tissue growing around streptothrix clumps.

PLATE XIII.

A. Rods, filaments and curious forms of streptothrix seen in sections of bronchi in the human cases. They are stained reddish brown by Gram's method, this reaction being due to iodine.

B. Streptothrix forms from 10 days' growth of 14th generation in bouillon stained red in hot carbolic fuchsin, the intense color being retained after decolorization of the specimen in 97% alcohol.

PLATE XIV.

Streptothrix colony surrounded by cells and organizing tissue in rabbit's lung. The bulbous ends of the filaments are deeply stained by Weigert's fibrin dye.

PLATE XV.

Obliteration of rabbit's bronchus by new growth of connective tissue, the plug being channeled with canals lined by bronchial epithelium. The new tissue appears œdematous. The section is from the lung of Rabbit No. 10, killed 37 days after intratracheal injection of broth culture of streptothrix (p. 183).

PLATE XVI.

Section of lung of Case I showing necrotic and suppurative bronchitis, with streptothrix clumps within the necrotic material in a bronchus. Fibrin and cells in the surrounding alveoli.

FIG. 5.

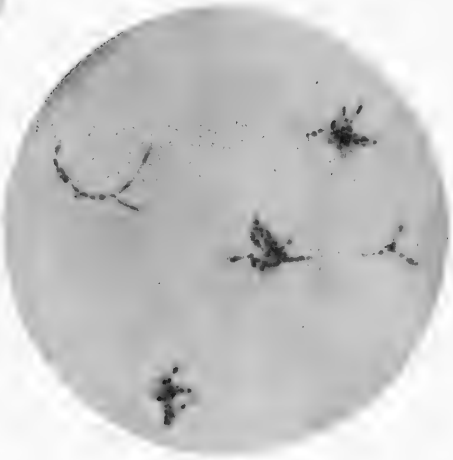

FIG. 4.

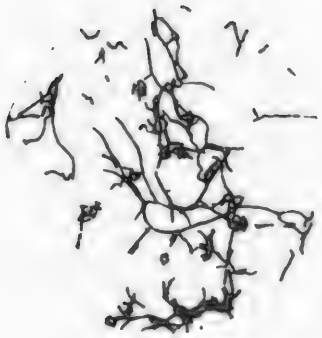

FIG. 3.

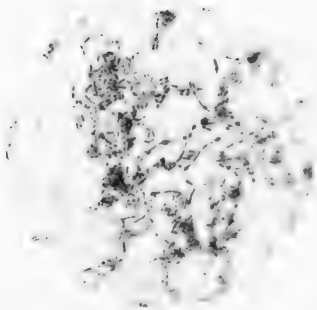

FIG. 2.

FIG. 1.

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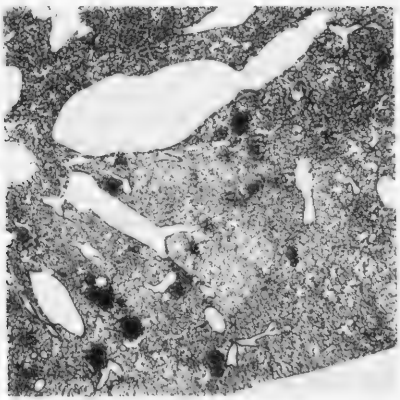

FIG. 8.

FIG. 7.

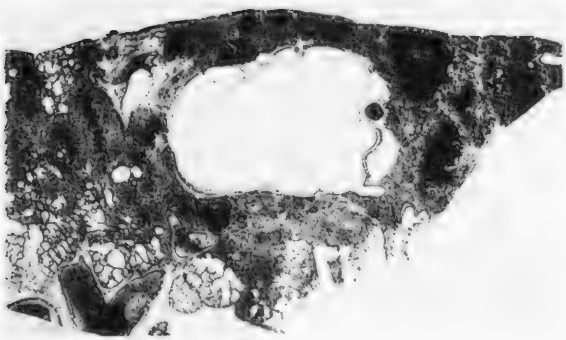

FIG. 6.

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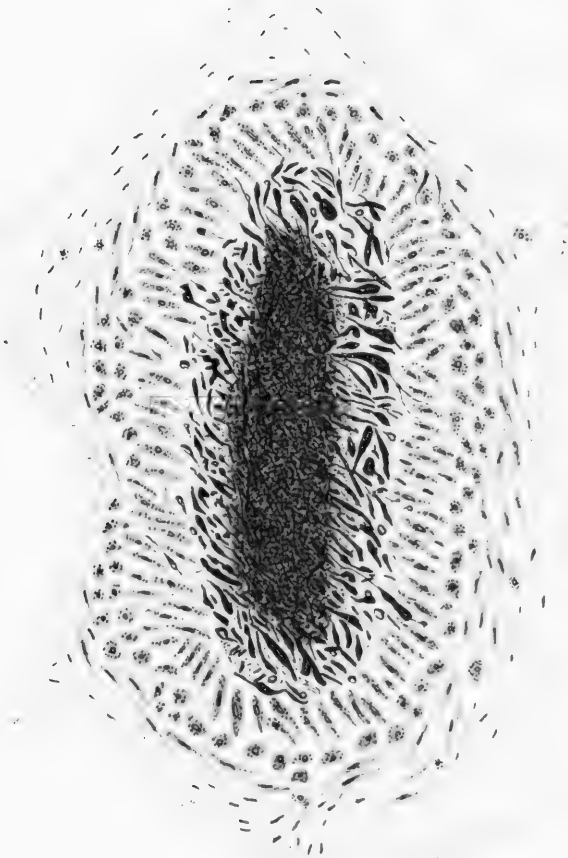

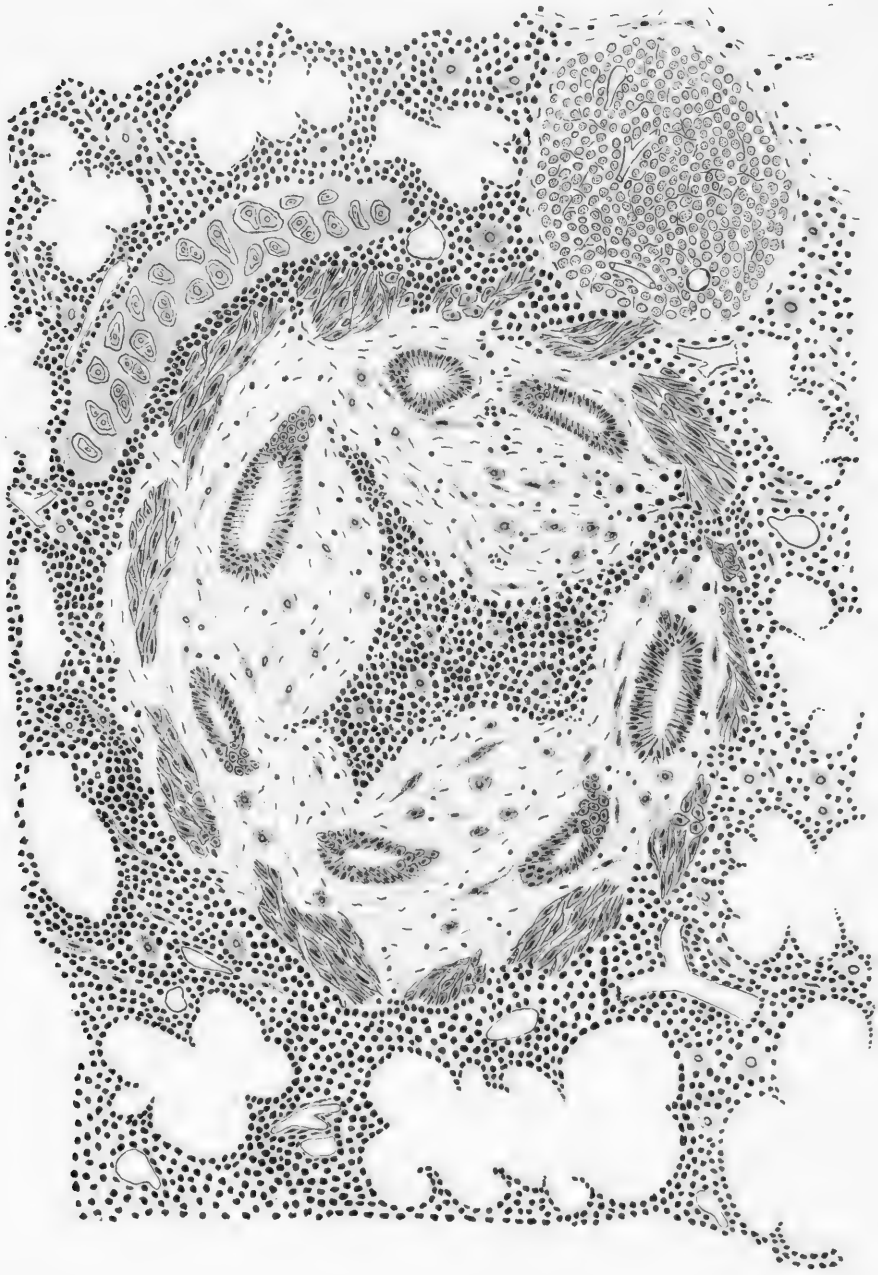

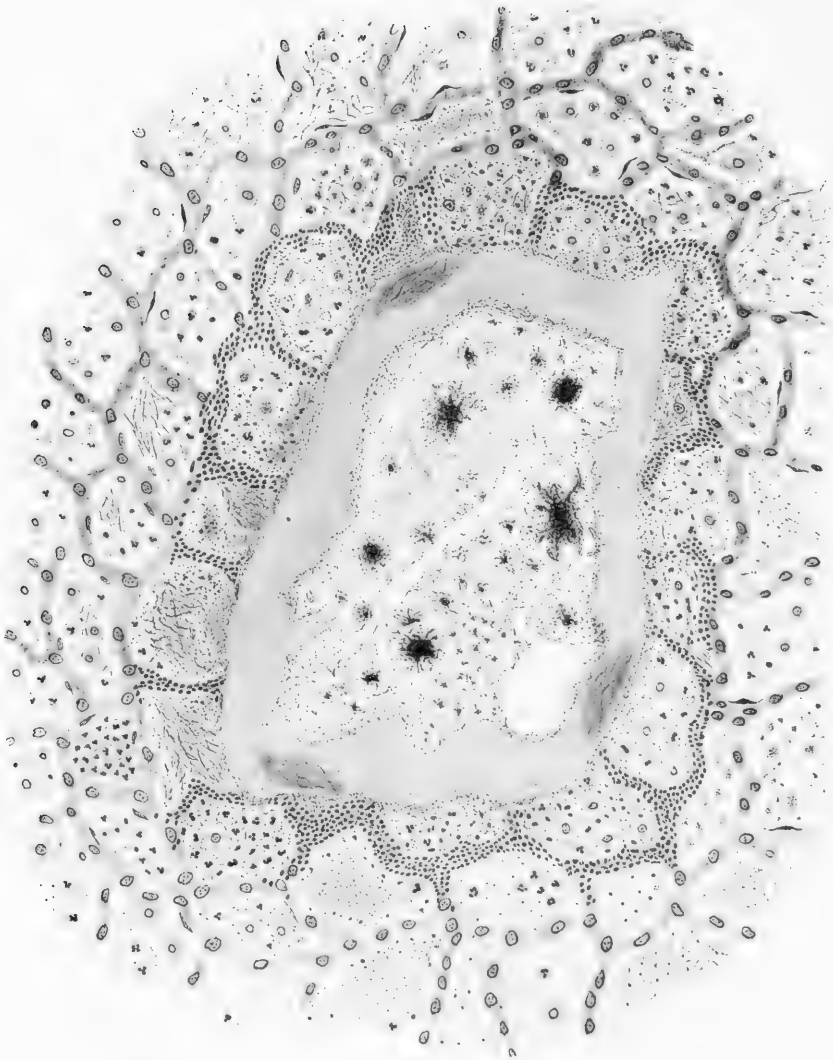

ACUTE INTERNAL HYDROCEPHALUS. A CLINICAL AND PATHOLOGICAL STUDY.[1]

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AND

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PLATES XVII AND XVIII.

Clinical History.—W. S., male, white, 33 years old, was admitted to the Philadelphia Hospital on April 15, 1899 complaining of great headache, general weakness and abdominal pain. His family and personal histories were unimportant, possibly except that four years before he had had typhoid fever. On April 13 he stopped work, complaining of violent headache and pain in the back of the neck. The pain continued throughout the night, and the following day he became delirious and was sent to the hospital.

Examination showed a muscular man with flushed face. He lay in bed with the head retracted and the legs strongly flexed upon the abdomen. The temperature was 102° F., pulse 82, and respiration 24. He was stuporous but could be roused by sharp questioning. He would answer a few questions properly and would then become incoherent. Physical examination revealed no abdominal or thoracic disease. There were no palsies. The head was in extreme extension and could not be flexed. Lateral movement of the head was also impossible on account of muscular rigidity. Attempts at passive movement caused severe pain. There was no muscular rigidity elsewhere. Sensation, so far as could be determined, was normal. He was too weak to stand. The tongue was brown, the lips cracked and the abdomen distended. The plantar jerks and knee jerks were absent even with reinforcement. The bladder and rectum were under control. The urine contained a trace of albumin, a few hyaline casts, and had a specific gravity of 1027. The red blood-corpuscles numbered 4,850,000, the white corpuscles 9000, and the hæmoglobin reached 75%. Widal's test was made repeatedly with

[1] Read at the meeting of the Philadelphia Neurological Society, December, 1899.

negative results. Various culture media inoculated with blood remained sterile. The tentative diagnosis was acute meningitis.

On April 22 Kernig's sign was present. Herpes of the lips and nose and a bluish mottling of the skin appeared. The mental state varied greatly. Sometimes the patient was restless and indeed quite violently delirious, at others stuporous, and again he would be almost normal, lying quiet, talking coherently and complaining only of headache and pain in the back of the neck. The pupils were dilated and slight lateral nystagmus was present. Slight deafness appeared. The plantar reflexes returned but the knee jerks remained absent. The eye grounds, examined by Dr. C. A. Oliver, were normal. On May 10 he was stone deaf. For several days he hiccoughed violently. The temperature which ever since his admission had been fluctuating between 101° and 103.4° F. now gradually dropped to normal and he improved greatly in all ways. Pain disappeared and the mind became clear and active. The knee jerks returned. Slight rigidity of the muscles of the neck persisted. His mental state was peculiar. He was perfectly coherent, could talk well and understood all that was said to him, but he was too well content with himself and his surroundings, he had no realization of his being sick and, though really too weak to stand, maintained that he was very well and would be out of doors in a day or two. He was trifling and jocose and, as his sister said, altogether unlike his usual self. He reminded one of a man in the beginning stage of paretic dementia. He gained greatly physically for a month and then the fever rose to 103° and the picture became the same as it was on admission. After a few days the fever again fell, and he again improved but for only a short time. On June 6 the temperature suddenly rose, the old symptoms returned, subsultus was added, nystagmus became marked and on June 9 he died.

Necropsy.—This was made the next day. The calvarium was normal. The dura was tense and elastic. The convolutions were broad, pale and flattened and the sulci were almost obliterated, appearing as lines beneath the pia. About the base over the cerebellum the pia was a little milky. The infundibulum was distended and pressed upon the optic nerves but not enough to distort them. On opening the ventricles a large amount of clear fluid escaped. All were dilated and the aqueduct of Sylvius was much wider than normal. The ependyma was boggy and separated easily from the underlying brain tissue. In the lateral ventricles it was roughened and at the tip of either inferior horn a band of white tissue stretched from the outer to the inner wall. The lining of

the fourth ventricle was somewhat boggy. The choroid plexus of the third ventricle was injected and œdematous, that of the lateral ventricles was rolled up into an oval mass the size of a hazel nut and adhered to the walls. The spinal cord was very wet and in the lumbar region were several small hernia-like protrusions of the white matter into the pia. The dura was a little thickened in the cervical region. The heart and lungs were normal. The right pleura was adherent. The spleen was cirrhotic and the kidneys showed a subacute parenchymatous nephritis.

Histological Examination.—On microscopic examination the choroid plexus of the lateral ventricles showed a small tumor-like mass which on cross-section consisted of a capsule surrounding meshes of blood-vessels. There were a large number of hyaloid bodies (Plate XVII, Fig. 2, A, E, B), often twenty in one field, staining deeply with hæmatoxylin but taking only a faint stain with Lugol's solution. There was marked infiltration of small round cells in the capsule (Plate XVII, Fig. 2, C) and foci of them scattered through the sections. Their nuclei stained deeply and were about half the size of those of the ependymal cells. The vessels were distended with red corpuscles. The choroid plexus of the third ventricle showed similar but slighter changes; that of the fourth was normal.

The ependyma of the third ventricle showed microscopically the same tendency to separate from the underlying tissue as was noted in the gross specimen. A marked ependymal proliferation was present in the lateral ventricles (Plate XVII, Fig. 1). The nuclei of the cells varied in size, shape, and staining properties. The ventricular surface was covered by an amorphous granular layer several times thicker than the ependyma and containing nuclei scattered through it. A golden yellow pigment was present in the ependyma of all the ventricles. The vessels beneath the ependyma to a depth of one-half centimetre were actively congested and were surrounded by round cells (Plate XVII, Fig. 1, D). These cells not only occupied the perivascular spaces but also extended some distance into the surrounding tissue. The greater number of them were small round cells with nuclei slightly smaller than the nuclei of glia cells and surrounded by a narrow ring of cell substance. Though the nuclei of the glia cells within the area of infiltration were swollen and possibly proliferating we feel confident that the larger number of these cells did not arise from the glia. Scattered among the smaller nuclei larger, faintly-staining nuclei resembling very closely those of the endothelial cells of the capillaries were occasionally seen. They were probably endothelial in origin but of this there is no direct evidence. No

polynucleated cells were present in the infiltration. Some of the large pale nuclei were irregular in outline but did not resemble the nuclei of polynuclear leucocytes in any other respect.

The neurogliar cells also showed proliferative changes. The nuclei of those immediately beneath the ependyma were at least twice the normal size and were surrounded by distinct cell bodies from which the processes radiated (spider cells). Further from the surface the nuclei became smaller, the protoplasm less, and the radiating processes finer until at a depth of about three quarters of a centimetre the tissue became normal. The area of change in the glia cells corresponded closely to that of congestion and round-cell infiltration.

The nature of the lesions suggested that they might have been caused by the reaction of the tissues to some toxic or irritative constituent of the ventricular fluid.

The cortical and spinal ganglion cells stained by Nissl's and Weigert's methods and by carmine showed nothing abnormal. The cranial nerves were stained by the Marchi method and carmine. The fourth and sixth showed a few black granules. The eighth pair were very much degenerated; the others normal. The spinal nerve roots showed only a few black granules in the lumbar posterior roots. The white matter of the cord showed no degeneration by the Marchi, Weigert, and carmine stains.

To sum up: A man is suddenly seized with fever, bradycardia, constipation, rigidity of the muscles of the neck, headache, stupor and delirium. After three weeks, during which the intensity of the symptoms varies greatly, he improves very much physically but shows many of the mental symptoms of paretic dementia. A week later fever and the meningeal symptoms return, last about a week, again intermit for four days only to return again and end in death. Post-mortem examination reveals only a moderate internal hydrocephalus, proliferation of the ependyma and ependymal glia, perivascular round-cell infiltration in the sub-ependymal tissues, and sclerotic and degenerative changes in the choroid plexus. What caused the lesions?

Internal hydrocephalus from mechanical causes is quite common, but idiopathic internal hydrocephalus is, or seems to be, rare and has attracted the attention of clinicians and pathologists only in recent years. Before the discovery of the tubercle bacillus it was quite

common to call cases of hydrocephalus tubercular even though tubercles were not present. Barthez and Rilliet[2] separated these from those manifestly tubercular, but the credit of having established idiopathic internal hydrocephalus as a distinct clinical and pathological entity belongs to Quincke.[3] There are two varieties, one acute, the other chronic but often having acute exacerbations.

The acute variety is most frequent in children. In adults it usually follows injury to the head, the infectious fevers, especially typhoid and pneumonia, and acute or chronic alcoholic poisoning. The onset is sudden with headache, delirium, photophobia, vomiting and retraction of the head—symptoms resembling the irritative stage of septic or tubercular meningitis from which affections it may be impossible to differentiate it. Fever, however, may be absent or not so high as in septic meningitis and may either quickly disappear or vacillate with the other symptoms. The headache and muscular rigidity are not so intense nor so constant and the delirium, instead of persisting, alternates with periods of normal consciousness. The intermittency of the symptoms and the early increase of intracranial pressure, shown by choked disc, paralytic mydriasis, and, in early life, enlargement of the head, are characteristic. Examination of the cerebrospinal fluid is important. In tubercular meningitis the bacillus is often, although not always, present in the fluid withdrawn by lumbar puncture, and in septic meningitis inoculations of culture media give characteristic growths, and the fluid is either turbid or distinctly purulent and contains endothelial cells, leucocytes, red corpuscles, and pus microorganisms. In idiopathic hydrocephalus the fluid is under higher tension than normal varying from 150 to 700 mm. (water manometer). It differs but slightly in its constituents from the normal, is clear and transparent, with a specific gravity of 1008 and contains albumin and sugar (?) in small quantities.

The chronic variety often follows the subsidence of the acute symptoms or, in children, may appear only with the increase in intra-

[2] Barthez and Rilliet. Traité clinique et pratique des maladies des enfants. Paris, 1861.

[3] Quincke, Volkmann's *Samml. klin. Vortr.*, 1893, n. F., No. 67, and *Deutsche Zeitschr. f. Nervenheilk.*, 1896, ix, p. 149.

cranial pressure caused by the union of the bones of the skull. A receding optic neuritis may be the only clinical manifestation. In other cases the symptoms may for a long time be vague and inconstant so that mere neurasthenia may be suspected. A large group present the symptoms of brain tumor. Optic neuritis is almost constant and transient or permanent bitemporal hemianopsia caused by varying pressure of the distended infundibulum on the optic chiasm may be present. Headache, vomiting, vertigo and local or general convulsions, and cranial nerve palsies are frequent. Localizing symptoms are almost never present except in the rare cases in which the hydrocephalus is confined almost entirely to the fourth ventricle and causes symptoms of cerebellar disease. The long course of the disease often ending favorably or seeming to do so only to recur, and the good effect of lumbar puncture point to the true nature of the disease, especially if the spinal fluid is found to be under high pressure at the time of puncture.

Our case in the early stages was diagnosed as a meningitis of the convexity and of the cord. Typhoid and spotted fever were both thought of but the continuous absence of the Widal reaction, the absence of spots, the non-enlargement of the spleen, and the clinical course excluded the former and the absence of eruption, of leucocytosis, of spinal-root symptoms, and the negative result of bacteriological investigation made the latter impossible. The peculiar variability of the symptoms, the afebrile intermissions, and the curious mental state made the diagnosis doubtful but it was retained as the best working hypothesis.

There are many points of clinical interest in the case. Kernig's sign which was at one time thought to be pathognomonic of meningitis was present during almost the whole course of the disease. The absence and subsequent return of the knee jerk and plantar reflex is also interesting. Absence of the knee jerk is not very uncommon in brain tumor but its return after an absence of three weeks without any lesion in the cord to explain it must be very rare. The absence could not have been caused by œdema of the cord because at autopsy that was very great though the reflex had returned several weeks before. We can only note the phenomenon, not explain it.

Central deafness with degeneration of both eighth nerves is not mentioned in any other case. It probably was caused by an unusual distribution of the pressure from the ventricles on the nerves at their exits. The optic nerves are most frequently affected and after them the sixth and seventh. In our case the optic nerves were normal save for a small focus of old degeneration near the centre.

The mental state may resemble paretic dementia. Indeed in Prince's[4] cases so close was the resemblance that that diagnosis was held for a time. Jocosity, a total neglect of the relative importance of things, and mild delusions of grandeur were the predominant factors in the mental attitude of our patient.

The pathological findings are interesting because in only a few of the cases heretofore reported have changes in the ependyma and subependymal tissues been described. Quincke[5] explains the absence of inflammatory changes in his cases by the subsidence of the primary hyperæmia subsequent to the occurrence of the hydrocephalus. The membranes of the brain may according to him be the seat of an acute serous inflammation like that which occurs in the pleura and the synovial linings of joints. In other cases the acute onset of the symptoms and their rapid disappearance make it seem probable to him that changes similar to those of angio-neurotic œdema may occur. In our case there were both recent and old changes in the choroid plexus. The proliferation of the interstitial tissue, the dilatation of the vessels, the round-cell infiltration, and the ependymal changes all pointed to an acute inflammation. The encapsulation of the plexus, the adhesions to the ventricular wall, and the bands across the tips of the ventricles indicated an old process. The large number of hyaloid bodies in the plexus of so young a man is evidence of degenerative change in the vessels leading to their obliteration and subsequent change into hyaloid material. All the steps in the process from hyaline degeneration of the media to complete obliteration, fragmentation, and calcification of the fragments are fairly well shown in the sections. That these bodies may have another and very

[4] Prince. *Journal of Nervous and Mental Disease*, 1897, xxiv, p. 473.
[5] Op. cit.

different origin, namely from proliferated endothelial cells blocking up the lymph spaces and undergoing hyaline degeneration, there is abundant evidence in the same sections, but the changes in the blood-vessels are the more important in connection with the other evidences of a chronic and acute inflammation in the plexus.

The changes in the ependyma correspond to those in the choroid plexus. The thickened ependymal membrane and the adhesions and roughened surfaces of the lateral ventricles were of long standing. The acute inflammatory changes were widespread. Some authorities[6] hold that since the ependyma consists only of a layer of cells without blood-vessels it cannot be the seat of acute inflammation, but the distended network of capillaries immediately beneath the ependymal layer, the perivascular round-cell infiltration, the amorphous exudate on the surface and the hypertrophic changes in the glia must be considered to prove the inflammatory nature of the process. The fact that these changes were confined almost wholly to the ependyma and extended only a few millimetres beneath, gives the impression that they were the results of a local reaction of the cerebral tissues to some toxic action of the ventricular fluid rather than an acute primary inflammation leading to a serous exudation. To determine if possible which of these two conditions was present and to discover the effect of toxines and acids on the ependymal membranes we made the following experiments:

Sterilized urine, glycerine extract of the adrenals (P. D. & Co.), tuberculin, hydrochloric and carbolic acid were injected into the ventricles of kittens by means of a large hypodermic syringe.

The three adrenal kittens died within twenty-four hours. A slight increase of the nuclei surrounding the vessels immediately beneath the ependyma and dilatation of the capillaries of the brain substance for a short distance were the only changes noted. In one kitten there was intense dilatation of the vessels of the choroid plexus with hæmorrhages into its meshes.

Three kittens injected with urine were killed after three, six, and ten days. The changes were the same in all, differing only in intensity

[6] Boenninghaus, Die Meningitis serosa acuta. Eine kritische Studie. Wiesbaden, 1897.

(Plate XVIII, Fig. 4). They consisted of a proliferation of the ependyma with an amorphous exudate upon the surface, swelling of the glia fibres which stained deeply, and a perivascular small round-cell infiltration, the whole resembling very much the condition found in the case here reported. In one specimen the layer of cells covering the choroid plexus failed to stain and looked very much like a fatty reticulum. In this specimen also a layer of round nuclei occupied the space immediately beneath the ependyma. Tuberculin gave similar but less marked results.

As dilute hydrochloric and carbolic acids produced the same effect we will describe the former only. The animals were killed at the end of a week. There was no excess of ventricular fluid. The ependyma looked normal and was not boggy or roughened. On microscopic examination (Plate XVIII, Fig. 3) the ependymal layer was intact, but the cells stained very faintly, the nuclei could hardly be seen, and the cell bodies were granular and their margins indistinct. Where this condition was most intense a granular layer covered the ependyma, evidently the debris of degenerated cells. Immediately beneath the ependyma and most marked on the under and inner surfaces of the ventricles a layer of round nucleated cells was present. They were four and five deep in certain areas and in others suddenly disappeared. They had small, round nuclei which stained deeply and were of the same size and character as those surrounding the deeper vessels. No polynuclear cells were found among them. Their probable origin was the network of capillary vessels beneath the ependyma. Columns of small nuclei surrounding the smaller vessels extended from this layer deeper into the brain substance. The glia network within the zone of capillary dilatation and perivascular infiltration was close meshed and had the appearance of a fibrillar structure running parallel to the ependymal surface. The columnar cells of the choroid villi were granular. The superficial cells were mere shadows and often the outer border was absent making the surface of the plexus look frayed. The nuclei were either absent or only outlined and failed to stain. The condition appeared to be caused by a proliferation of the cells; the outer layer becoming degenerated and finally breaking down into an amorphous mass resting upon the surface of the other cells.

We conclude from these experiments that the non-purulent inflammation of the ependyma produced by acid irritants differs only in degree from the reactive changes following the injection of toxines into the ventricles. Changes in the ependyma without changes in

the subjacent tissue probably do not occur. The inflammatory condition experimentally produced, by whatever agent, did not cause any increase in the ventricular fluid and the only evidence of an exudate from the ependyma was the amorphous material which probably was made up of degenerated cells.

The microscopic sections in the toxine experiments resembled the sections from the case reported and to that extent confirm the opinion that the changes found were secondary to a toxic condition of the ventricular fluid. The clinical history offers other evidence in support of this view. For example the mental condition of the patient, corresponding to that seen in other auto-intoxications, would be best explained by a such an hypothesis. The exacerbations which Quincke compares in their sudden development and variability to angio-neurotic œdema appear to us to be rather the manifestations of varying intensity of auto-intoxication, such as occurs in uræmia and syphilis. Finally the hydrocephalus alone by its mere mechanical action, if sufficient fluid is present, can cause many symptoms.

DESCRIPTION OF PLATES XVII AND XVIII.

PLATE XVII.

FIG. 1. Section of the floor of the lateral ventricle. Stained with hæmatoxylin-eosin. The marked irregularity of the floor is shown—caused partly by the folding of the ependyma (*C*), partly by the amorphous exudate on the surface (*B*), and partly by the hypertrophy of the sub-ependymal glia (*A*). The zone of perivascular small, round-cell infiltration (*D*) is seen extending some distance beneath the ventricular surface.

FIG. 2. Section of the choroid plexus of the lateral ventricle. *C*, the capsule very rich in nuclei. Hyaloid bodies in different stages are seen at *A*, where the hyaline change with calcification is beginning in a vessel, and at *B* and *E* has advanced to irregular hyaloid forms. The vessels of the plexus contain many leucocytes which are very rich in a granular pigment (*D*).

PLATE XVIII.

FIG. 3. Showing the reactive changes in the ependyma and choroid plexus of a cat after the injection of hydrochloric acid (5%) into the ventricle. *A*. Granular degeneration of ependymal cells. *B*. Layer of round nuclei immediately beneath the ependyma. *C*. Perivascular infiltration of round cells extending deeper into the brain substance.

FIG. 4. Ventricular surface of the brain of a cat after injection of sterile urine. *A*. Granular degeneration of ependyma cells. *B*. Sub-ependymal layer of round nucleated cells. *C*. A vessel surrounded by an accumulation of round cells. The blood-vessels deeper in the tissue are not affected.

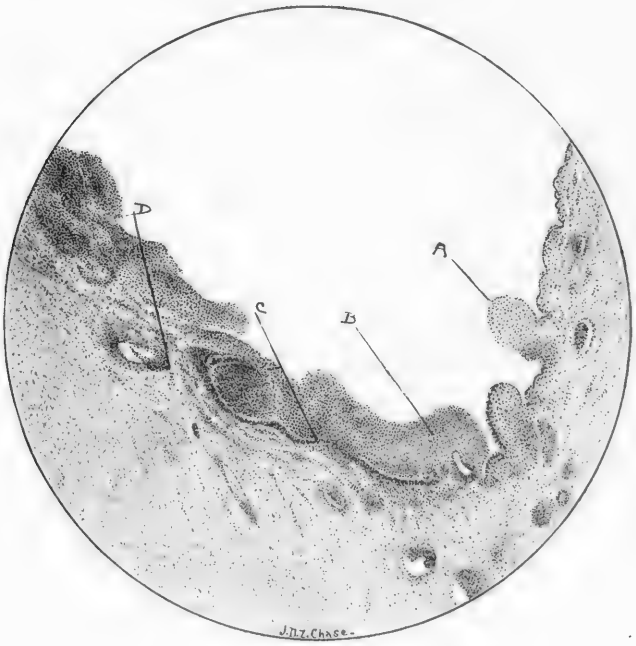

FIG. 1.

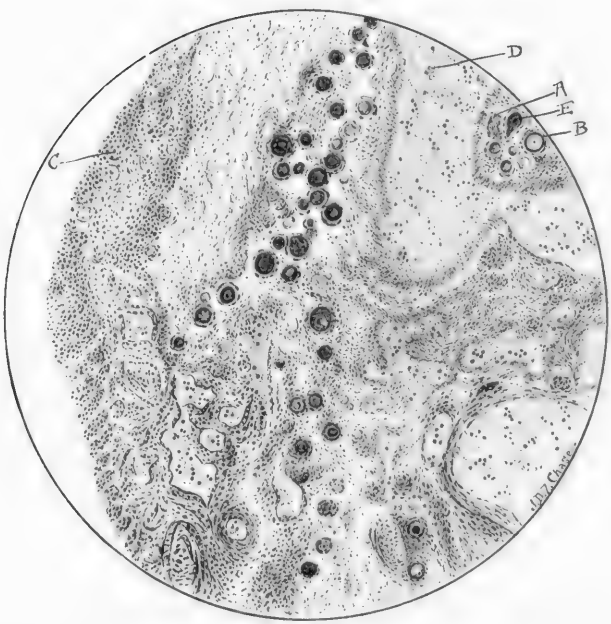

FIG. 2.

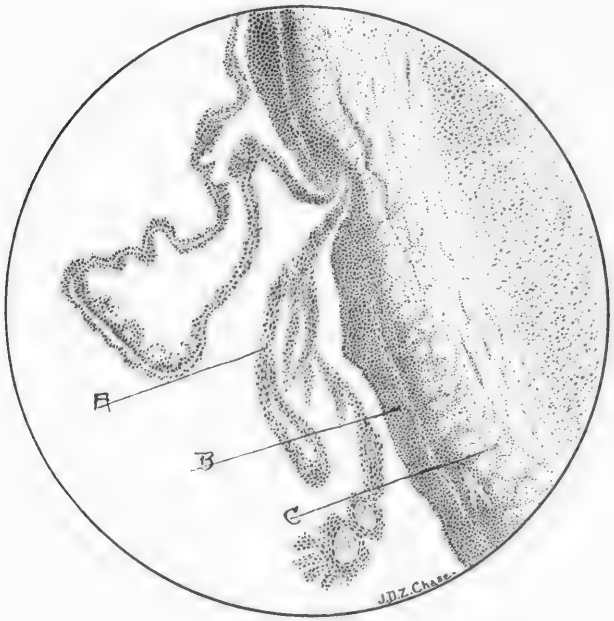

FIG. 3.

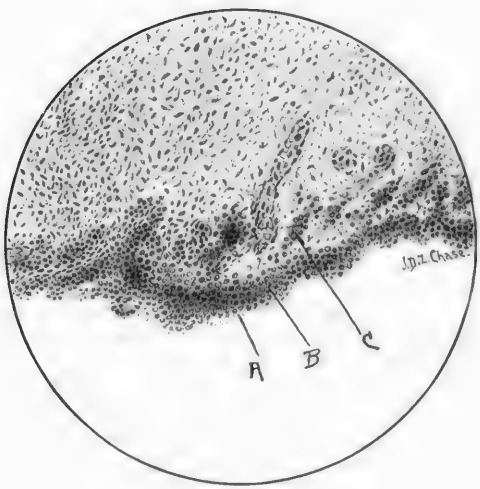

FIG. 4.

A PRELIMINARY REPORT ON ACID-RESISTING BACILLI, WITH SPECIAL REFERENCE TO THEIR OCCURRENCE IN THE LOWER ANIMALS.[1]

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At the suggestion of Professor Dock, I began four years ago to make some investigations on the occurrence of the smegma bacillus in man. While pursuing this work, which is not yet completed, it occurred to me that it might be not only of interest but of importance to know whether the smegma bacillus or bacilli resembling those of tuberculosis existed to any great extent in the lower animals. It is this point I wish to consider here.

Resistance to decolorization by strong acids (Säurefestigkeit), as was first shown by Ehrlich, is the most characteristic staining reaction of the tubercle bacillus and, at the date of its discovery, differentiated this bacillus from all other known bacteria except the bacillus of leprosy. Since this time, however, a number of other acid-resisting bacilli have been discovered, the list increasing with especial rapidity during the last few years. Soon after the discovery of the tubercle bacillus in 1882, G. Zahn[2] noted the presence in non-tuberculous sputum of bacilli resembling the tubercle bacillus in staining reaction, and recently acid-resisting bacilli resembling tubercle bacilli have been found in the sputum in cases of pulmonary gangrene by A. Fraenkel,[3] Pappenheim,[4] and Rabinowitsch.[5] Laabs[6] and Moëller[7] have also recorded the presence of acid-resisting bacilli in non-tuberculous buccal secretion and sputum.

[1] Read at the meeting of the Medical Society held in the Medical College, Ann Arbor, December 14, 1899.

[2] *Med. Cor.-Bl. d. württemb. ärztl. Vereins*, 1884, liv, p. 129.

[3] *Berliner klin. Wochenschr.*, 1898, pp. 246; 880.

[4] Ibid, 1898, p. 809.

[5] *Deutsche med. Wochenschr.*, 1900, p. 257.

[6] Inaug.-Diss., Freiburg i. Br. 1894.

[7] *Zeitschr. f. Hyg.*, 1899, xxxii, p. 211.

The prototype and longest known of these acid-resisting pseudo-tubercle bacilli is the smegma bacillus first described in 1885 by Alvarez and Tavel[8] and by Matterstock[9] and Bitter,[10] and obtained in artificial culture by Laser[11] and by Czaplewski.[12] The occurrence of acid-resisting bacilli in the human intestine is mentioned by Crämer,[13] de Giacomi,[14] and von Jaksch,[15] so that there is proof that bacilli resembling in tinctorial properties and often more or less closely in morphology the tubercle bacillus may appear in the secretions from the human genito-urinary, intestinal and respiratory tracts. The acid-resisting bacillus cultivated by Czaplewski[16] from a case of leprosy is probably not identical with the bacillus of leprosy. Dietrich[17] reports an interesting observation of the presence of acid-resisting bacilli resembling the tubercle bacillus in a suppurating ovarian cyst which had ruptured into the intestine, their presence, together with the symptoms, leading to the erroneous diagnosis during life of tuberculous peritonitis.

An especial incentive to the search for acid-resisting bacilli has been the detection by Petri and by Rabinowitsch of such bacilli in butter and by A. Moëller of similar bacilli in timothy grass and dung. Since the first demonstration of tubercle bacilli in butter by Brusaferro[18] in 1890, this hygienically important subject has been investigated by several writers with most discordant results, the percentages of samples of butter in which the tubercle bacillus was found varying from 0 to 100. Obermüller[19] examined 14 samples of butter from a single source in Berlin, all of which he claims contained the tubercle bacillus. Rabinowitsch,[20] who examined butter from a number of shops in Berlin and in Philadelphia, demonstrated the tubercle bacillus in all the samples from one of the largest Berlin houses, whereas all of the other samples were free from this bacillus. In a considerable proportion of the samples,

[8] *Progrès méd.*, 1885, 2. s., ii, p. 135, and *Arch. de phys. norm. et path.*, 1885, 3. s., vi, p. 303.

[9] *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1885, p. 65.

[10] Virchow's *Archiv*, 1886, cvi, p. 209.

[11] *Münch. med. Wochenschr.*, 1897, p. 1191.

[12] Ibid, 1897, p. 1192.

[13] *Sitzungsb. d. phys.-med. Soc. zu Erlangen*, Dec. 1882.

[14] *Fortschr. d. Med.*, 1883, i, p. 145.

[15] Klinische Diagnostik innerer Krankheiten. Wien and Leipzig, 1896.

[16] *Centralbl. f. Bakter.*, 1898, xxiii, pp. 97, 189.

[17] *Berliner klin. Wochenschr.*, 1899, p. 189.

[18] *Baumgarten's Jahresbericht*, 1890, vi, p. 271.

[19] *Hyg. Rundschau*, 1897, vii, p. 712.

[20] *Zeitschr. f. Hyg.*, 1897, xxvi, p. 90, and *Deutsche med. Wochenschr.*, 1899, p. 5.

however, she found bacilli morphologically and tinctorially resembling the tubercle bacillus. Butter containing these bacilli, injected into the peritoneal cavity of guinea-pigs, produced nodules superficially like tubercles and containing the bacilli, but distinguishable from true tubercles by their histological characters. Injection of pure cultures of the bacilli did not produce tubercles. Rabinowitsch says that Koch in 1896 recognized the occurrence in butter of acid-resisting bacilli resembling the tubercle bacillus. Shortly before Rabinowitsch's description of these bacilli in butter, there appeared, as a comment upon Obermüller's disquieting observations, a brief statement concerning the detection of these pseudo-tubercle bacilli by Petri,[21] who published in the following year[22] a fuller description in his study of the question of tubercle bacilli in butter.

The publications of Rabinowitsch and of Petri have led to further investigations concerning the presence of acid-resisting bacilli resembling the tubercle bacillus in butter, among which may be mentioned those of Hormann and Morgenroth,[23] Grassberger,[24] Herbert,[25] Weissenfeld,[26] Ascher,[27] Coggi, [28] and Korn.[29] While the results vary as to the frequency of these bacilli in butter, some indeed being negative, and also to some extent as to their characters, it is established that bacilli morphologically and tinctorially like tubercle bacilli have been repeatedly found in butter, and their occurrence should lead to great care in the diagnosis of suspected tubercle bacilli in this material, as well as in milk. Culturally these bacilli can be readily distinguished from the tubercle bacillus, and the same distinction can usually be made without much difficulty in their pathogenic properties.

Grassberger inoculated the peritoneal cavity of 20 guinea-pigs with Vienna market butter and noticed in 10 of these characteristic anatomical changes. Firm whitish yellow masses were found on the peritoneum. These masses were made up of a delicate fibrinous frame-work with remnants of butter and large numbers of bacilli within. The bacilli stained red when treated with carbolic fuchsin and decolorized in the nitric acid

[21] *Hyg. Rundschau*, 1897, vii, p. 811.
[22] *Arb. a. d. k. Gesundheitsamte*, 1898, xiv, p. 1.
[23] *Hyg. Rundschau*, 1898, viii, pp. 217; 1081.
[24] *Münch. med. Wochenschr.*, 1899, Nos. 11 and 12.
[25] *Arb. a. d. path.-anat. Institut zu Tübingen*, 1899, iii, p. 207.
[26] *Berliner klin. Wochenschr.*, 1899, p. 1053.
[27] *Zeitschr. f. Hyg.*, 1899, xxxii, p. 329.
[28] *Giorn. d. R. Soc. Ital. d'Igiene*, 1899, No. 7.
[29] *Arch. f. Hyg.*, 1899, xxxvi, p. 57, and *Centralbl. f. Bakt*, 1899, xxv, p. 532.

solution. He obtained pure cultures of this so-called butter bacillus, but like Rabinowitsch he failed to produce lesions in guinea-pigs by their inoculation. If, however, he mixed the culture with sterilized butter or sterilized paraffin and inoculated the mixture into guinea-pigs, the animals died and on post-mortem examination showed the characteristic nodules described above. Sterilized butter or sterilized paraffin produced no lesions when inoculated. If preparations of this organism were left for 24 hours in alcohol and ether they no longer resisted the decolorizing solution. Grassberger found no genuine tubercle bacilli in the samples of butter examined. He further observed that if guinea-pigs were inoculated with Kretz's bacillus of timothy grass lesions similar to those produced by the butter bacillus occurred.

In her first publication on this subject Rabinowitsch stated that Capaldi in examining her specimens was impressed with the resemblance between these butter bacilli and acid-resisting bacilli which he had found in cow's dung. Severin[30] had previously reported the existence of such bacilli in horse's dung and Ferran[31] in cow's, horse's and human feces. Of especial interest and importance are the recent observations of Alfred Moëller[32] who has cultivated from vegetable fodder and from the dung of different animals bacilli which he designates respectively as the dung bacillus, the timothy bacillus or grass bacillus I, and the grass bacillus II. These bacilli of Moëller are even more resistant to decolorization by acids than the tubercle bacillus, which they also resemble morphologically, but are distinguished from the latter by cultural characteristics. Especially noteworthy are Moëller's statements concerning the production of nodules closely resembling genuine tubercles by inoculation into guinea-pigs of pure cultures of these bacilli. Moëller speaks of confirmation or control of his results by Czaplewski, Kretz, and Lubarsch.

There can be no doubt that different species or varieties are represented among the various acid-resisting bacilli described by the investigators cited as "smegma bacilli," "butter bacilli," "dung bacilli," "timothy" or "grass bacilli," etc., but further work is needed to elucidate the differences or relationships between the members of this interesting group of bacteria.

[30] *Centralbl. f. Bakter.*, Abth. II, 1895, i, p. 97.

[31] Abst. in *Centralbl. f. Bakter.*, 1897, xxii, p. 484.

[32] *Deutsche Med.-Ztng.*, 1898, p. 135; *Deutsche med. Wochenschr.*, 1898, p. 376; *Therap. Monatsh.*, 1898, xii, p. 607, and *Centralbl. f. Bakter.*, 1899, xxv, p. 369.

As already stated, the special object of my investigation was to determine to what extent acid-resisting bacilli are to be found on the bodies of animals, a subject which has received hitherto but little attention. The occurrence of such organisms in animal products was also considered, but to a less extent. Inoculation and culture experiments are under way and these results will be published later. In my work I adopt the name acid-resisting bacillus in preference to the too loosely used term smegma bacillus. It remains to be proved whether there is any relationship of the smegma bacillus to similar bacilli found on parts of the body other than the genitals, and to some of those found in animal products. Moreover the question has been raised as to whether the so-called smegma bacillus as it is seen in man, is a distinct species or whether the term represents a group of closely allied organisms.

The material for this investigation was obtained by scraping the parts with a blunt instrument. At times, as in the case of soft smegma, the material was easily obtained, and in that case a small bit was crushed between two cover glasses. In the case of dry epithelial scales, the scrapings were rubbed up with a drop or two of sterile water and with this suspension coverslip preparations were made and allowed to dry in an incubator. The slips in all cases were fixed by passing three times through the flame. They were all stained with hot carbolic fuchsin, after which they were thoroughly treated with fresh 25 per cent nitric acid solution, washed in water, and counterstained in Loeffler's or aqueous methylene blue. From three to six slips were thus prepared from each specimen and examined. The instruments used were sterilized before each examination. Fifty-five animals and a few samples of milk and feces were examined.

Horse.—The smegma of the horse exists in large amount; 10 to 20 grammes, or more, may be obtained from the sheath at one time. It is of a grayish-black color and is tolerably firm. The smegma of the mare is quite soft, of a light gray color and is found in small amount. The bacilli found in horse's smegma are for the most part slender, clean-cut rods and resemble very much the tubercle bacillus. Other forms may at times predominate, thus in the scrapings

from the vulva of one mare there was found on one slip a large number of capsulated bacilli which remained red after decolorizing in acetic acid. The smegma of one horse, two mares, one Shetland horse and one Shetland mare were examined, with positive results in all.

Dog.—The smegma of the dog, in the cases examined, was a creamy, semi-fluid, yellowish substance, existing in small amount. In this substance bacilli precisely resembling the tubercle bacillus may be found. Many beautiful beaded organisms were seen in some preparations. In the yellowish discharge so commonly seen on the penis of dogs similar bacilli were seen. The combination of these bacilli with the many leucocytes, mucus, and other microorganisms which abound, make a picture difficult to distinguish from that of tuberculous sputum. In this discharge, which apparently comes from the urethra, numbers of large phagocytes may be seen, but without bacilli. Four dogs were examined with positive results in three, negative in one.

Cow.—The cows used for this experiment were those of a dairy farm. There was no evidence of external disease of the teats or udders; aside from an occasional wart, they were to all appearances normal. These cows had not been subjected to the tuberculin test. The material for the examination was obtained by scraping the moistened teat or udder with a scalpel. The scrapings—dust, epithelial cells, etc.—were allowed to fall on bits of moistened filter paper placed in small tin boxes; coverslip preparations were made as already described.

It was not difficult to demonstrate acid-resisting bacilli in these scrapings. Generally speaking, these bacilli were much thicker than the tubercle bacillus. In some slips several different forms may be seen, as bent rods, rods with irregular outline, beaded forms, and club-shaped bacilli. In one specimen there were a number of slender bacilli, sometimes slightly bent, which stained a distinct but light pink, some isolated and scattered through the field, others in groups of five or more. These bacilli precisely resemble the tubercle bacillus. On account of the large amount of epithelium which takes the carbolic fuchsin deeply and resists decolorizing solutions, special

care was taken to expose these specimens for a considerably longer time than is necessary for the decolorization of sputum. Some slips were left in the acid solution until no further trace of red was visible to the naked eye, and still in these preparations red bacilli could be demonstrated. The bacilli were usually seen free from the epithelial cells. In every cow examined large numbers of yeast cells and spores which stained red were found. The habit so prevalent of lubricating the hands of the milker with the first strippings undoubtedly accounts for the invariable presence of yeast cells. The sugar in the milk favors the growth of these organisms.

In all, eight cows were examined for acid-resisting bacilli with positive results in five, negative in three. The so-called glair or vaginal mucus of one cow gave a negative result.

Eight preparations were made from the sediment obtained from a centrifugal separator. Each specimen showed many acid-resisting bacilli, some of which might easily be mistaken for the tubercle bacillus.

Guinea-pig.—The material for this examination was obtained from the glans penis, prepuce, vulva, anus, and from the mammæ of both male and female. Although the prepuce is quite long and completely covers the glans, yet the latter as a rule is very dry and only at times can a slight collection of a yellowish white material or smegma be seen. This substance has an odor similar to that of human smegma. The vulva which has no deep folds gave material which looked like slight epithelial desquamation mixed with dirt. All told, five male and five female guinea-pigs were examined. In all the males, acid-resisting bacilli were found on the genitals. On the mammæ of one and on the anus of another similar organisms were demonstrated. All the females but one gave positive results. The mammæ of two of these were examined with positive results in one. The organisms usually found were shorter and thicker than the tubercle bacillus. Occasionally longer and more slender forms were seen on the mammæ. Not infrequently large groups of red bacilli can be seen on a faded pink epithelial cell.

Rabbit.—The glans penis, prepuce, vulva, and perineal pouches were examined. The genital organs themselves were in all cases

very clean. The perineal pouches, situated on either side of the genitals, contain a large collection of a yellowish, soft, granular, oily material having a peculiar penetrating odor—the rabbit odor. This material microscopically is seen to be made up of numerous oily droplets, of squamous epithelial cells, which are more or less swollen, and of many kinds of bacteria. Notwithstanding the large amount of oily material in the perineal pouches, in the ten rabbits examined not a single acid-resisting bacillus was found. This fact is of interest in view of the common belief that fat is necessary for the staining reaction of the smegma bacillus. The fat in smegma, however, generally exists in a saponified form. The mammæ of five rabbits were also examined but with negative results.

Cat.—In the cat, as in the rabbit, the genitals are not exposed, and are very clean, and it is with difficulty that a cover-glass smear can be made. A few bacteria which stain blue are all that can be seen. Two male cats were examined with negative results.

White Rat.—On the prepuce when retracted, and at times on the external surface near the meatus, there may be seen small yellowish semisolid lumps about the size of a common pin head. Microscopically this material contains swarms of very short, thick, acid-resisting bacilli. These may be seen in groups of one hundred or more, or scattered singly through the field. A simple examination of these acid-resisting bacilli shows that several varieties or species are present. Thus some resemble diplococci, others have a drumstick form, while others have a central, clostridium-like enlargement. Seven males were thus examined with positive results in five, negative in two. The female shows no special material or smegma, and it is only with the greatest persistence that any material is obtained. The vagina at times contains a mucous substance. Three females examined showed positive results in one, negative in two.

The feces of the rabbit, guinea-pig, and white rat was examined with negative results.

Many are accustomed when speaking of the smegma bacillus, to think of it as a typical organism like the tubercle bacillus. This is not correct inasmuch as we have to deal with a group of bacilli which differ markedly among themselves. As in man, so in the lower ani-

mals, we find in the smegma various forms of microorganisms which in spite of comparatively long exposure to acid solutions retain their color, whereas the other bacteria with which they are associated decolorize readily. The extreme variation in the form of these acid-resisting bacilli clearly indicates a difference in species.

General Morphology.—The smegma of the lower animals very often contains slender, clean-cut, slightly bent rods which frequently show a distinctly beaded arrangement, and in this respect are not to be disintinguished from the tubercle bacillus. On the other hand, smegma bacilli are met that are appreciably shorter or longer than the tubercle bacillus. Moreover, irregular forms can be observed, the bacilli instead of being straight may be bent in the middle; this bend may be scarcely perceptible (comma bacillus), or may be almost a right angle. At times the stain is taken up unevenly and as a result the bacilli show irregular borders.

The occurrence of acid-resisting diplococci or diplo-bacilli and of drumstick forms has been already referred to. Oval, yeast-like organisms, capsulated bacteria, short threads of from four to six cells, and spores are met which retain the stain the same as the ordinary smegma bacillus. The organisms found on parts of the body other than the genital organs, as on the teats of the cow, usually vary in form as much as those in smegma.

Decolorization.—In the resistance which the smegma bacillus and other forms referred to offer to decolorization by acids, these organisms resemble the tubercle and leprosy bacilli. At one time the tubercle bacillus was supposed to have a specially impermeable cell wall, but this view has gradually given way to the belief that the peculiar reaction is due more to the composition of the cell contents than to the cell wall proper. That the resistance of the smegma bacillus to decolorization is not inconsiderable is seen in the fact that specimens of smegma can be left in 25 per cent nitric acid solution for ten minutes without the least decolorizing effect. The same is true of the bacilli found in the scrapings from the cow. Invariably the specimens will resist exposure for three to five minutes to this acid solution, or to the same strength of sulphuric and of acetic acids. An exposure of ten seconds to absolute alcohol decolorizes the bacilli

in the majority of cases, but at times a much longer exposure has practically little decolorizing effect.

As has been stated, some of the acid-resisting bacilli which are met among the lower animals resemble in form the tubercle bacillus. In the staining properties and especially in the resistance to decolorizing solutions this resemblance is rendered more striking. Nevertheless it would be a gross error to conclude that the tubercle bacillus was actually present. It is quite possible that the real tubercle bacillus may have been present, but its recognition by the only crucial tests—cultures and growth in the animal body—was not resorted to, for the reason that the immediate object in view was to demonstrate the presence of acid-resisting bacilli on the lower animals.

The presence of acid-resisting bacilli in milk or in butter may mislead and doubtless has misled those who depend entirely upon the recognition of the tubercle bacillus by its morphological and staining properties. Reports of the presence of tubercle bacilli in milk and in butter based on such observations are by no means few. The mere detection of such bacilli in milk or butter is now recognized as giving no support to the conclusion that they are tubercle bacilli. In order to identify such acid-resisting bacilli it is necessary to resort to cultures and animal experiments, but even here a certain amount of caution must be observed, not to mistake nodules produced by injection of butter and pseudo-tubercle bacilli for genuine tubercles.

CONCLUSIONS.

The results obtained in this study may be briefly summarized:

1. Acid-resisting bacilli are found in many of the lower animals, more especially the horse, cow, dog, guinea-pig and white rat. In the case of the rabbit and cat no such organisms were detected.

2. Many of these acid-resisting bacilli resemble the tubercle bacillus and the smegma bacillus of man.

3. The acid-resisting organisms are undoubtedly of different species and there is good reason to believe that the term smegma bacillus denotes not a definite species but rather a group of bacilli having common staining properties.